MODIFIERS OF TUMOROUS HEAD GENES IN NATURAL POPULATIONS AND LABORATORY STOCKS OF DROSOPHILA MELANOGASTER

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THE tumorous head (tu-h) abnormality and genetic mechanism were discussed by GARDNER (1948), GARDNER and WOOLF (1949) and NEWBY (1949). Penetrance, expressivity and relative viability were investigated by GARDNER and RATTY (1952). The influence of high and low temperatures on the expression was described by GARDNER and WOOLF (1950). Modifiers and alleles of tumorous head genes were found in laboratory stocks by GARDNER and WOOLF (1949) and GARDNER and STOTT (1951). The purpose of the present paper is to report the results of experiments designed to determine the presence, nature and frequency of modifiers for the tumorous head genes in natural populations and laboratory stocks.

MATERIALS AND METHODS

The material used for this study was a stock of flies (*Drosophila melanogaster*) carrying genes for an hereditary abnormality characterized by irregular growths in the head region. Outcrosses between tu-h females and males from laboratory stocks produced about 30 percent of abnormal flies in the F_1 at 22°C. Reciprocal outcrosses between tu-h males and females from laboratory stocks, other than Oregon R, Crimea and Stephenville, produced less than one percent of abnormal F_1 flies. Small percentages, not exceeding five percent of abnormal flies, were obtained in the F_2 from both reciprocal crosses except in the presence of modifiers (DEARDEN 1949; GARDNER and STOTT 1951). This pattern was used as a standard for comparison with the results of outcrosses involving natural populations and laboratory stocks suspected of carrying alleles or modifiers of tu-h genes.

Flies from natural populations were collected in the field with nets and traps. Following are listed the habitats and locations from which the eight field collections were obtained: I, tomato field, Edinburg, Texas; II, tomato field, Palisade, Colorado; III, picked peaches, Provo, Utah; IV, tomato field, Santa Clara, Utah; V, garbage can, St. George, Utah; VI, peach orchard, Kanosh, Utah; VII, peach orchard, Kanaraville, Utah; VIII, apple orchard, Veyo, Utah. In most cases large samples including several hundred flies were collected. The flies were transported to the laboratory and maintained in half-pint milk bottles containing the standard cornmeal, agar, and molasses media. Males taken at random from the original collections were mated with tu-h females. Virgin females taken as they appeared from the first generation of

cultured collections were crossed with tu-h males. Single pair matings were used in all experiments. Between 100 and 300 flies were classified from each mating.

The temperature was controlled within ± 1°C. Unless otherwise indicated the cultures were maintained at 25°C. The percentages of abnormal flies were summarized with accuracy to the nearest whole number. Percentages smaller than 0.5 were recorded as 0.

EXPERIMENTAL DATA

Modifiers of tu-h genes in natural populations

The results of reciprocal pair matings between tu-h flies and those collected from natural populations are summarized in table 1. Flies from the Canton

TABLE 1

Summarized results of reciprocal pair matings between tu-h flies and those collected from natural populations.

Cross	F_1		F_2				
	N[1]	Avg. % Abn.	N	No.[3] 0–5% Abn.	No. 10% Abn.	No. 30% Abn.	No. 50% Abn.
tu-h[2] × Canton	25	30	25	25	0	0	0
Canton × tu-h	25	0	25	25	0	0	0
tu-h × I	5	37	5	5	0	0	0
I × tu-h	6	0	6	3	3	0	0
tu-h × II	12	23	12	5	2	3	2
II × tu-h	26	0	26	19	4	3	0
tu-h × III	23	25	22	10	6	6	0
III × tu-h	40	7[4]	30	16	10	4	0
tu-h × IV	17	39	17	15	2	0	0
IV × tu-h	24	0	24	22	2	0	0
tu-h × V	22	36	22	17	4	1	0
V × tu-h	24	0	24	14	9	1	0
tu-h × VI	16	34	16	14	0	2	0
VI × tu-h	23	0	21	11	9	1	0
tu-h × VII	5	30	5	5	0	0	0
VII × tu-h	13	0	13	9	4	0	0
tu-h × VIII	7	40	7	7	0	0	0
VIII × tu-h	10	0	10	7	3	0	0

[1]Number pair matings.
[2]Female parent always written first.
[3]Number pair matings with 0–5 percent of abnormal flies.
[4]Alleles of *tu-1* present.

stock were used as a check for comparison with those being tested for the presence of modifiers. Results of reciprocal crosses involving Canton were similar to those with another Canton stock (Canton Special) and seventeen other "wild" laboratory stocks reported previously (GARDNER and STOTT 1951). An average of about 30 percent of the F_1 progeny from the 25 pair matings between tu-h females and Canton males were abnormal. The F_1 progeny from the 25 reciprocal matings were all normal. The F_2 results were

similar for both reciprocal crosses. Less than one percent of abnormal flies was obtained.

The main difference between the results of the crosses involving flies from natural populations and those reported previously from laboratory stocks was the lack of uniformity among pair matings suggesting the segregation of genes in the natural populations. The laboratory stocks were apparently homozygous for the modifiers they carried. This might be expected as a result of the inbreeding which follows the usual procedure of maintaining laboratory cultures. In contrast, the natural populations carried modifier genes in different frequencies, presumably at equilibrium.

Modifiers in laboratory mutant stocks

The stock containing aristaless, dumpy, dachs, black, curved plexus and speck on one second chromosome and Curly and speck on the other (*al dp d b c px sp /Cy sp*) gave results (GARDNER and WOOLF 1949) which deviated from the

TABLE 2

Percentages of abnormal flies in the F_1 and F_2 from reciprocal crosses between tu-h and laboratory stocks suspected of carrying modifiers of tumorous head. Crosses with Canton were used as controls. The temperature was maintained at 21° C.

Cross	Second chromosome present	F_1		F_2	
		N[2]	%[3]	N	%
tu-h[1] × al dp d b c px sp/Cy sp	al ...	91	26	1855	8
	Cy ...	89	29	823	12
al dp d b c px sp/Cy sp × tu-h	al ...	194	1	980	11
	Cy ...	157	1	779	14
tu-h × al dp d b c pd sp/Cy		279	32	1326	10
al dp d b c pd sp/Cy × tu-h		282	0	683	7
tu-h × dp		995	8	905	12
dp × tu-h		682	2	1400	13
tu-h × dp°		251	42	821	4
dp° × tu-h		258	1	760	4
tu-h × dp°²		719	33	581	2
dp°² × tu-h		181	0	255	0
tu-h × c		993	16	955	3
c × tu-h		926	0	1190	4
tu-h × a px sp²		1132	9	931	0
a px sp² × tu-h		477	0	900	0
tu-h × b (pr) c px sp		215	14	171	1
b (pr) c px sp × tu-h		214	0	184	0
tu-h × b		424	21	954	0
b × tu-h		1030	0	1315	1
tu-h × Canton		1023	21	1288	0
Canton × tu-h		852	0	1504	0

[1]Female parent always written first.
[2]Total flies.
[3]Percentage of abnormal flies.

usual F_2 pattern. From both reciprocal crosses involving this stock about 10 percent of abnormal flies was obtained in the F_2. These results suggested that a modifier was carried in this laboratory stock. The results of reciprocal crosses with this and other related stocks are summarized in table 2. The table also includes the results of crosses with other stocks containing *dp*, *dp*^{o}, *dp*^{o2}, *c*, *a px sp*^{2}, and *b*. Control crosses between tu-h and Canton are given for comparison.

The F_1 results of most crosses listed in table 2 were within the expected range at 21°C. Percentages of abnormal flies from crosses between tu-h females and males from the *dp* and *a px sp*^{2} stocks were lower than expected. The maternal effect was indicated for all reciprocal crosses. The F_2 results were fairly similar for both reciprocal crosses involving tu-h and *al dp d b c px sp /Cy sp*. Approximately the same percentages of abnormal flies were observed among the progeny receiving the second chromosome containing the *Cy sp* genes (12 percent) and those receiving the *al dp d b c px sp* second chromosome (8 percent). These percentages of abnormal flies were considerably higher than those of the controls (0 and 0) suggesting the presence of a homozygous modifier in this stock. A similar stock, undoubtedly derived from the same source, with purpleoid (*pd*) substituted for plexus (*px*) gave similar results. Similar F_2 results were also obtained from crosses involving the stock carrying *dp* alone. These three stocks all carry modifiers which influence the basic genetic mechanism controlled by *tu-3*. The other stocks tested did not produce F_2 results significantly different from the controls.

To demonstrate more clearly the action of the modifier in combination with different numbers of *tu-3* genes, reciprocal backcrosses were made from the F_1 to both parent stocks (tu-h and *al dp d b c px sp/Cy sp*). The results are summarized in table 3. The percentages (16, 19, 17 and 24) of abnormal flies from the backcrosses between F_1 females from both reciprocal P_1 crosses and

TABLE 3

Results of backcrosses involving tu-h and al dp d b c px sp/Cy sp and Canton at 21° C.

Parents (P_1)		Second chromosome present	Backcross to tu-h				Backcross to other stock			
			$F_1 \times$ tu-h		tu-h $\times F_1$		$F_1 \times P_1$		$P_1 \times F_1$	
Female	Male		N	%	N	%	N	%	N	%
tu-h	al... / Cy sp	al...	246	16	963	22	152	1	241	4
		Cy sp	236	19	126	32	255	0	341	0
al... / Cy sp	tu-h	al...	939	17	504	39	370	2	168	7
		Cy sp	423	24	194	38	809	1	551	8
tu-h	Canton		895	0	1005	29	1192	0	262	0
Canton	tu-h		717	0	796	32	914	0	688	0

tu-h males were similar, and considerably greater than those from the controls (0). Three *tu-3* genes were involved in this cross and presumably one modifier. The modifier therefore was dominant in this genetic environment. Three *tu-3* genes were also involved in the control crosses but no modifier came from the Canton stock. The maternal effect was indicated in the results of backcrosses between tu-h females and F_1 males. The results were like those of the controls. The lower percentages and greater irregularities among the results of backcrosses between the F_1 flies and flies from the *al dp d b c px sp/Cy sp* stock were attributed to the segregation of a single *tu-3* gene. Three modifiers presumably were involved in this cross but only one *tu-3*. In the control backcrosses no modifiers were present and only one *tu-3*. The results of the control crosses indicate that the independent action of *tu-3* in the genetic environment provided by the Canton stock is very slight.

SUMMARY

Collections were made from eight natural populations of *Drosophila melanogaster*. Flies from these collections were tested for the presence of modifiers of the tumorous head genes (*tu-1* and *tu-3*). All populations were found to carry modifiers of *tu-3*. Two different types of modifiers were distinguished on the basis of percentage of abnormal F_2 flies from outcrosses with tumorous head. Both types were detected in four of the eight samples. These modifiers were present in different frequencies in the different populations. The main difference between these results involving natural populations and those reported previously from laboratory stocks was the lack of uniformity among the results of pair matings involving natural populations, suggesting a segregation of genes. The inbred laboratory stocks were apparently homozygous for the alleles and modifiers they carried while different frequencies were indicated in different wild populations. Three laboratory stocks which probably originated from the same source were each interpreted to carry the same modifier of *tu-3*. Six other stocks carrying some of the same mutant genes were free from this modifier.

ACKNOWLEDGMENTS

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THE EFFECT OF INTENSITY OF INFRARED ON X-RAY INDUCED CHROMOSOME ABERRATIONS IN TRADESCANTIA

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PREVIOUS investigations have shown that infrared is effective in increasing the number of chromosomal aberrations induced by X-radiation (KAUFMANN, HOLLAENDER and GAY 1946; SWANSON and HOLLAENDER 1946; YOST 1951). There has been little agreement at the interpretive level, however, on the mode of action of the infrared in producing this increase. Two hypotheses have been advanced. One proposes that infrared radiation affects only the process of recombination of broken ends of the chromosomes, i.e., that recombination is favored at the expense of restitution (KAUFMANN, HOLLAENDER and GAY 1946; KAUFMANN and WILSON 1949). The other postulates that the effect is to increase the total number of primary breaks (SWANSON and HOLLAENDER 1946). Recently, evidence has been presented which favors the latter of these two hypotheses (YOST 1951). In an attempt to explain the mechanism whereby the infrared radiations exert their effect on Tradescantia chromosomes, it was proposed that, in addition to complete breaks realized at the time of radiation, the X-rays also induce potential breaks which become complete and available for recombination upon the addition of infrared energy in posttreatment. It was further postulated that the effect of pretreatment is to weaken the chromosome structure, thus making it more susceptible to fragmentation by X-radiation (SWANSON 1949; YOST 1951).

If the preceding series of postulates be true, a decrease in the intensity of the infrared treatments, keeping the total dose constant, should result in a decrease in the number of exchange aberrations recovered in posttreatment, with no decrease in the number of terminal deletions and isochromatid aberrations. This follows from the assumption that the breaks involved in producing the increase are not complete until the addition of the infrared; and since the lower the intensity, the lower the probability that any two complete breaks will be coincident in time in the same cell, the possibilities of an exchange taking place in any cell are lowered. On the other hand, there should be no effect of intensity of infrared in pretreatment, since there is no disjunction of the chromosome structure which can undergo recombination, with infrared treatment alone. These tests imply that recombination can take place whenever broken ends are available, but the length of time a broken end can remain open is limited in Tradescantia. Previous data have shown that infrared posttreatments are effective in Tradescantia as long as 96 hours after X-radiation

[1] This work was done at the Johns Hopkins University as a part of the thesis requirement for the doctor's degree.

(YOST 1951). The only way in which the recombination hypothesis developed for the Drosophila data could explain the Tradescantia data would be under the assumption that there is no time limit to the recombination of broken ends in the plant cells. Therefore, the intensity study seems to provide a conclusive test of the potential break hypothesis and the experiments to be reported here were designed with this in mind. In addition, dosage curves in the infrared are presented which help to elucidate the infrared problem.

EXPERIMENTAL PROCEDURE

Inflorescences of *Tradescantia paludosa,* ANDERSON and WOODSON (clonal line number 5 of SAX) were used as the experimental material for this study. X-ray treatments consisted of radiation from an industrial type of instrument,

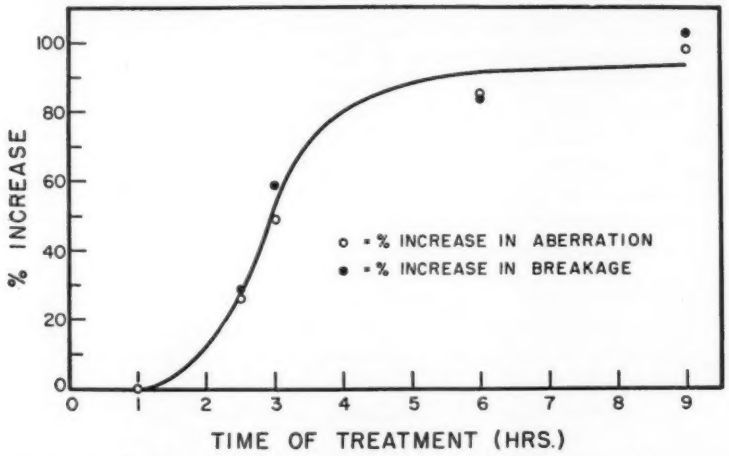

FIGURE 1.—The relationship between dosage of infrared postreatment and the percentage increase of aberrations.

delivered at 80 kilovolts and 10 milliamperes, the distance from the tungsten target being 16 inches. The window of the tube provided filtration equivalent to 1.5 mm of aluminum. The treatment time was one minute for all exposures. The dosages, in roentgen units, are given with the data.

Buds to be treated with infrared were placed in a pyrex tube, which was rotated slowly in a beam of light emitted by a commercial, 250 watt drying lamp operated at 110 volts. The glass tube was cooled by a stream of water to eliminate any possibility of a general temperature rise in the buds. In the intensity studies, the radiation was filtered through a Corning No. 2540 glass filter. The intensity of the infrared radiation was varied by changing the distance between the source and the buds. Measurements of the intensity were made with an Eppley thermopyle, this instrument being calibrated against a standard lamp so that absolute values for the intensities might be obtained.

The values for the intensity of the radiation are given with the data for each experiment. The dosage of infrared given to the buds was kept constant by increasing the time of exposure in proportion to the decrease in intensity. The dosage curve (figure 1) for the infrared posttreatment effect was made with the bulb in the position which gave the higher intensity reading measured for the intensity experiments. The dosage curve for pretreatment (figure 2) was made with a water filter (1 cm, inside dimensions) interposed between the source and the Corning filter. Since it is known that the water and glass do not absorb appreciably in the region which is effective, the differences in duration of treatment necessary to produce comparable effects may be ascribed to differences in the intensity of the infrared radiation.

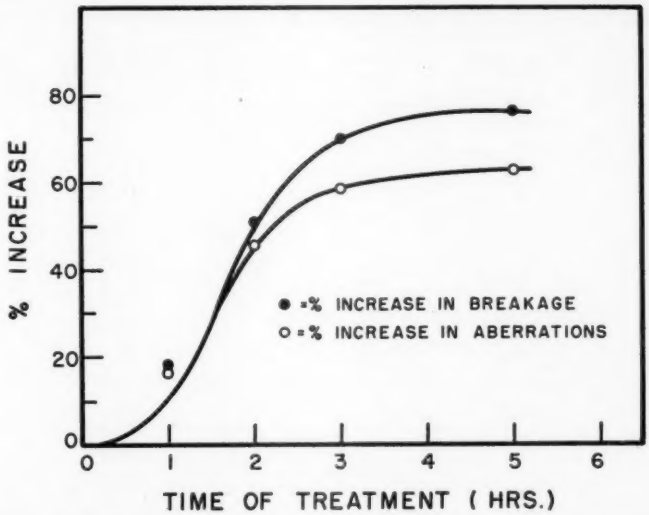

FIGURE 2.—The relationship between dosage of infrared pretreatment and the percentage increase of aberrations.

In each experiment, all buds, whether treated with infrared or not, were X-rayed at the same time to insure adequate controls. The material was fixed in 3 : 1 alcohol-acetic, 20–22 hours after X-radiation, regardless of the time of infrared treatment. All aberrations scored at this time are chromatid aberrations, and only metaphase figures of the first microspore division were scored. Each line in the column headed " Chromosome Number " in the tables represents an analysis of 3 to 9 slides of acetocarmine smear preparations.

RESULTS

The dosage curves in tables 1 and 2 and figures 1 and 2 cannot be considered to represent any rigorous relationship. There is, under the present experimental conditions, too much fluctuation in the effectiveness of the infra-

red in inducing increases in breakage to permit the construction of a strict dosage-relationship. The curves were drawn to suit the points, but no attempt was made to fit the curves by statistical methods. The reasons which led to a choice of shape for the curve are evident from a consideration of the two curves and the two sets of data. The curve in figure 1 is not drawn through the first set of points as a lag period is apparent in figure 2. Unpublished data indicate that a lag period is also present in this case. The data do not permit a distinction to be made between the two curves at present. There can be little

TABLE 1

The relationship of induced increase of aberrations to dose of infrared radiation when given as a posttreatment.

Infrared intensity: 56×10^4 ergs/cm²/sec.			X-ray dose: 68 r			
Treatment duration (hrs.)	Chromosome number		Single deletions	Isochromatid deletions	Exchanges	% Incr. (S.D. & E.)
0	3828		170	236	45	
		%	4.45	6.15	1.18	
1	3390		176	259	27	
		%	5.20	7.65	1.38	17.1
		χ^2	2.38	6.31	0.70	2.82
		P	0.18	0.015	0.42	
2	2874		176	216	59	
		%	6.13	7.52	2.02	45.8
		χ^2	9.75	5.10	8.41	
		P	0.005	0.03	0.007	
0	3600		169	262	49	
		%	4.70	7.28	1.36	
3	3600		234	320	110	
		%	6.51	8.90	3.06	57.8
		χ^2	11.12	6.28	23.90	
		P	0.002	0.015	<0.001	
0	1800		117	197	27	
		%	6.51	10.94	1.50	
5	1800		167	221	67	
		%	9.28	12.29	3.72	62.5
		χ^2	9.56	1.56	17.56	
		P	0.005	0.25	<0.001	

doubt that the shape of the curve is correct. The curves were constructed on the basis of the percentage of increase of single deletions and exchange aberrations. Throughout the paper the isochromatid aberrations have not been included in any calculation of the overall percentage of increase associated with a particular infrared treatment, as the isochromatid aberrations apparently represent a distinct category of aberrations and show a rather erratic response to supplementary infrared treatments.

The curves are presented to show that there is a threshold dose and an easily achieved maximum. There can be little doubt that this maximum is real.

At the X-ray dosage employed, an analysis of the infrared-treated cells can be made with assurance. The maximum is not a function of scoring difficulty brought about by the induction of complex and unanalyzable aberrations. Nor is it the simple conversion of single-hit aberrations into multiple-hit types. The curves indicate that the maximum is present whether the number of aberrations or the number of breaks is used as a criterion of effect. It appears that

TABLE 2

The relationship of induced increase of aberrations to dose of infrared radiation when given as pretreatment.

Infrared intensity: 70.5×10^3 ergs/cm²/sec. X-ray dose: 50 r

Treatment duration (hrs.)	Chromosome number		Single deletions	Isochromatid deletions	Exchanges	% Incr. (S.D. & E.)
0	3408		99	164	24	
		%	2.91	4.72	0.70	
1	2190		56	101	10	
		%	2.55	4.61	0.46	0
		χ^2	0.57	0.15	1.36	
		P	0.46	0.70	0.27	
0	1470		30	67	19	
2½	1530		37	83	22	25.8
						$\chi^2 = 1.44$
0	3600		75	148	19	
		%	2.06	4.11	0.53	
3	3600		100	190	40	
		%	2.78	5.28	1.12	49.1
		χ^2	3.64	5.46	7.54	9.30
		P	0.07	0.03	0.008	
0	3900		80	182	34	
		%	2.05	4.67	0.87	
6	2848		109	164	45	
		%	3.83	5.76	1.54	85.0
		χ^2	18.74	4.03	7.05	
		P	<0.001	0.05	0.009	
0	1800		36	83	14	
		%	2.00	4.61	0.78	
9	1800		69	101	30	
		%	3.83	5.62	1.67	97.8
		χ^2	10.67	0.98	5.89	16.78
		P	0.003	0.33	0.02	

there is a definite upper limit to the number of possible aberrations induced by a given dose of X-radiation, which cannot be exceeded by the addition of large, or even excessive, quantities of infrared radiation. Since infrared alone is without visible effect on the chromosomes, this is to be expected. A comparison of the two figures reveals that the shape of the curve is independent of the intensity of infrared radiation, the duration of the treatment, and the dose of X-rays.

INTENSITY RELATIONSHIP

Reference to figure 2 will show that an exposure of two hours of infrared is on the "straight line" portion of the dosage curve. This exposure was chosen for the intensity studies since it was felt that this portion of the dosage range should be the easiest to evaluate in terms of percentage increase. All pretreatments were given immediately before X-radiation; all posttreatments were given immediately after X-radiation. The data presented in table 3 show that a definite effect on exchange aberrations is achieved when the intensity of infrared radiation is lowered by a factor of 3.3 in posttreatment. There is no effect of intensity upon the single-hit types of aberration, with the exception that the number of single deletions may be increased slightly. That is, there is an increase of 38 percent in single deletions when the intensity of radiation

TABLE 3

The relationship of induced increase of aberrations to the intensity of infrared radiation when given as posttreatment.

Treatment duration (hrs.)	Chromosome number		Single deletions	Isochromatid deletions	Exchanges
Infrared intensity: 56×10^4 ergs/cm²/sec.				X-ray dose: 68 r	
0	3828		170	236	45
		%	4.45	6.15	1.18
2	2874		176	216	59
		%	6.13	7.52	2.02
		χ^2	9.75	5.10	8.41
		P	0.005	0.03	0.007
Infrared intensity: 16.8×10^4 ergs/cm²/sec.				X-ray dose: 68 r	
0	3144		120	277	39
		%	3.82	8.81	1.24
6.6	3540		275	365	41
		%	7.77	10.30	1.16
		χ^2	33.96	4.32	0.10
		P	<0.001	0.04	0.93

used is high; there is an increase of 103 percent in the single deletions in the lower intensity series, on the other hand. It would appear that a posttreatment with infrared can increase the frequency of breaks, but that at the lower intensity their coincidence in time is lessened to such a degree that illegitimate reunion is precluded. The breaks therefore restitute or remain open as single deletions. If this interpretation be correct, the time limit for a break remaining in a rejoinable state is relatively short.

The data in table 4 show that there is no effect of lowering the intensity on any of the types of aberrations when infrared is given as a pretreatment.

DISCUSSION

The essential point of departure for the two hypotheses advanced to account for the action of infrared is whether or not the infrared causes an increase in

breakage *per se*. This difference of interpretation arose originally from the differences in response of Drosophila and Tradescantia to infrared radiation. Pretreatments are effective in both species, but posttreatments have not been shown to be effective in Drosophila, although they are in Tradescantia. Secondly, although gross chromosomal rearrangements are increased in Drosophila, dominant lethals (presumed to be structural rearrangements for the most part) are not. KAUFMANN and his co-workers have reasoned that if there were a total increase in the primary breaks there should be an increase in dominant lethals and recessive lethals. Since no such increase has been found, the recombination hypothesis gains weight. On the other hand, it has been shown that in Tradescantia the infrared treatments may be delayed until long after the time limits of recombination processes, as determined by several

TABLE 4

The relationship of induced increase of aberrations to the intensity of infrared radiation when given as pretreatment.

Treatment duration (hrs.)	Chromosome number		Single deletions	Isochromatid deletions	Exchanges
Infrared intensity: 56×10^4 ergs/cm²/sec.				X-ray dose: 68 r	
0	3000		142	231	37
		%	4.74	7.70	1.23
2	3180		219	292	70
		%	6.89	9.19	2.20
		χ^2	12.82	5.37	8.57
		P	<0.001	0.03	0.007
Infrared intensity: 16.8×10^4 ergs/cm²/sec.				X-ray dose: 68 r	
0	4752		207	376	70
		%	4.36	7.90	1.46
6.6	3714		251	349	85
		%	6.76	9.41	2.29
		χ^2	23.57	5.84	8.18
		P	<0.001	0.02	0.007

investigators (cf. LEA 1946), have elapsed (YOST 1951). This fact supports the breakage hypothesis of SWANSON (1949).

Consideration of the facts presented above reveals that there is no real discrepancy. Consider first the argument that the lack of effect of posttreatment in Drosophila refutes the recombination hypothesis. If the effect of the infrared be upon recombination processes alone, then one would expect that the posttreatments rather than the pretreatments would be most effective, since the breaks are present after X-raying and not before, and since recombination does not take place in Drosophila sperm until after fertilization. There is some indication, however, that posttreatment effects can be achieved in Drosophila (GLASS 1950). The argument from dominant lethals is somewhat different. As KAUFMANN and WILSON (1949) have pointed out, there is a distinct similarity between the behavior of dominant lethals in Drosophila and that of

isochromatid aberrations in Tradescantia. The data which have been presented by SWANSON (1949) and those which are presented in this paper indicate that increases in isochromatid aberrations can be achieved, but that they fail in any one case to be highly significant. It can be noted from the data presented in tables 1 and 2 that there is no increase in isochromatid aberrations with a maximum dose of infrared. That is, in the 5 and 9 hour treatment series the P values are 0.25 and 0.33 respectively. Taking all the data together which are presented in this paper, one may state that an increase in isochromatid aberrations is achieved; however, the fluctuations of the increase of the isochromatid aberrations shown by the data do not permit any lumping of the data. The data presented in this paper show that in no case is there a highly significant increase of isochromatid aberrations resulting from infrared treatment. These data represent an analysis of 70,000 chromosomes, so that it is clear that isochromatid aberrations represent a special category, being relatively insensitive to infrared posttreatments and variably sensitive to pretreatments. Since dominant lethals may be largely isochromatid aberrations (DEMEREC and FANO 1944; FANO 1941), the absence of any significant increase in dominant lethals is not too surprising, especially if we consider that Drosophila chromosomes are less sensitive to X-ray breakage than Tradescantia chromosomes. Such considerations have led KAUFMANN and WILSON (1949) to the conclusion that primary breaks may be divided into two groups: complete ("thoroughgoing") breaks, types in which attachment of sister ends takes place immediately after X-radiation; and potential breaks, types which undergo the usual recombination processes in Drosophila at the time of fertilization. It is this latter category of potential breaks which may be influenced by infrared radiation to recombine rather than to restitute.

SWANSON (1949) has used the term "potential break" to describe the condition of the partially damaged chromosome resulting from radiation. There is a distinct difference between these two "potential break" ideas. SWANSON has proposed that the chromosomes are sometimes incompletely broken by X-radiation and that the absorbed infrared energies are sufficient to cause completion of these breaks with a consequent increase in the total number of primary breaks. The potential break, in this case, refers to loci of the chromosome in an activated state (YOST 1951; SWANSON and YOST 1951), and the remainder of this discussion will be carried out with the use of the term "activated state" to distinguish this hypothesis from that advanced as the potential break hypothesis for Drosophila. KAUFMANN and WILSON (1949) indicate that the potential break is a complete break, but that recombination of the broken ends is held in abeyance and may be influenced by the infrared. It is clear that either hypothesis may explain the data in both groups provided that there is an equivalence of dominant lethals and isochromatid breaks, or if it be assumed that the effective period of recombination in Tradescantia is longer than previous data have indicated. If the first assumption be made, there is little reason to prevent application of the activated state hypothesis to the

Drosophila data. If the second assumption be made, one may apply the recombination hypothesis to the Tradescantia data with equal validity, as the best evidence against this hypothesis rests on the extension of the period of posttreatment until after the period of recombination (SWANSON 1949; YOST 1951).

The data presented here, however, permit a distinction to be made between the two hypotheses. If the recombination hypothesis can successfully explain the Tradescantia data, the effect should be related to the total dose of infrared given to the buds. This follows since it is only possible to apply the recombination hypothesis if it be assumed that the period during which breaks remain open in Tradescantia is of 96-hour duration, and spreading the dose over 6.6 hours instead of 2 hours should be without effect. On the other hand, if the activated state hypothesis be correct, lowering the intensity of infrared should decrease the number of exchange aberrations without decreasing (and, in fact, possibly increasing) the number of single deletions. That is, if there be an increase in the total number of primary breaks induced by infrared radiation, and the restitution-recombination process is not interfered with, a significant decrease in the intensity of infrared radiation should spread the time of origin of the primary breaks resulting from posttreatment over a sufficiently long period so that there would be little or no opportunity for recombination to form exchange aberrations. This should result in a relative increase in the total number of single-break aberrations, with little or no increase in the multiple-break types. The data presented in table 3 show this to be the case. In this regard, it should be noted that the percentage of exchange aberrations induced by combined treatments, with high intensity infrared, was 2.02, as contrasted to a percentage of 1.18 induced by X-ray alone. On the other hand, when the infrared intensity was dropped by a factor of 3.3 the percentage of exchange aberrations induced by combined treatments was 1.16, slightly, though not significantly, lower than the percentage induced by X-rays alone, 1.24.

Taken alone the intensity data do not prove the activated state hypothesis. The intensity data merely support the conclusions derived from other experimental evidence to the effect that chromosome breaks lose their ability to recombine within a limited time in Tradescantia. The recombination hypothesis does not satisfy both the extension of pre- and posttreatment to 96 hours from the time of X-radiation, and the apparent limitation of the duration of open breaks shown by the intensity data presented here.

The lack of effect of reducing the intensity of infrared radiation when used as a pretreatment remains to be considered. It has been pointed out, from considerations of the types of aberrations resulting from combined treatments, that the effect of infrared radiations is to weaken the chromosome structure (YOST 1951). Recent data which show the infrared effect to be temperature sensitive indicate that the infrared radiations induce a metastable state in the chromosomes (SWANSON and YOST 1951). The activated loci, which have a semipermanent status and do not decay appreciably with time, have a higher

probability of becoming primary breaks than do unactivated loci when X-radiation energy is introduced into the cell. Therefore there should be no effect of lowering the intensity of the infrared pretreatment, as long as the total dose is kept constant. The data in table 4 strongly support this hypothesis.

It can be seen that there is no significant difference in the percentage increase induced with high or low intensities of infrared. At the high intensity an increase of 78 percent was attained in exchange aberrations. At the low intensity, the increase was 57 percent. Both increases are highly significant. There seems little reason to assume a significant difference between these increases, although the low intensity gave a slightly lower increase than the high intensity. The increases induced with low intensity radiation in pretreatment are average, and quite different in character from the lack of response shown by the low intensity treatment in posttreatment. It might be suggested that lower intensity radiation fails to achieve the highest levels of increase due to a " leakage " of energy from partially activated loci. That is, a higher intensity radiation has a greater probability of inducing all possible metastable states than a lower intensity radiation. This in no way alters the conclusions drawn from the complete absence of effect in posttreatment. These considerations are supported by the data presented as dosage curves. Here, too, the maximum increase at the lower intensity is lower than the maximum reached with high intensity infrared radiation.

Extension of the activated state hypothesis to explain the data obtained from Drosophila seems quite permissible, if the equivalence of dominant lethals and isochromatid aberrations is granted. Indeed, it is possible to extend the present hypothesis to Drosophila and disregard the equivalence of these aberrations. It is quite possible that these data refute the initial assumption that most dominant lethals are chromosomal aberrations.

It is possible to object to an extension of the activated state hypothesis to the Drosophila data on the grounds that it does not explain the recessive lethal data obtained by KAUFMANN and GAY (1949), who found no increase in the number of recessive lethals induced when infrared was used as a pretreatment. It is true that a number of recessive lethals have been found to be associated with chromosome breaks. There is no proof, however, that there is a clear-cut causal relation involved between breakage and lethal mutation although the question has been raised (cf. LEA 1946). HERSKOWITZ (1951) has calculated that 73.7 percent of the observed lethals are of breakage origin. Such a figure would suggest that substantial increases would be obtained with infrared treatment. New techniques have made possible a reinvestigation of this problem, and preliminary results indicate that the problem needs to be reopened. Such work is being carried on in this laboratory at the present.

As indicated earlier, the dosage relationship shown in this paper cannot be considered rigorously established. The nonlinear shape of the curves, however, is important in that it illustrates the fact that the infrared effect is not a " single-hit " phenomenon. This being the case, one might expect that changing

the intensity of radiation over a wide enough range might prevent the infrared from having an effect. This would be so if it were assumed that the absorbing moiety must reach a definite energy level within a rather restricted period of time. That is, if the trapped energy from the infrared "leaked off" faster than it was accumulated, sufficient energy to raise the sensitive structure to a metastable state might never be attained. The curves presented here indicate that the shape of the curve and the final maximum value attained are not directly related to the intensity of radiation used. However, as pointed out earlier, the possibility of some decay of the effect at lower than maximum intensity remains. The data presented by SWANSON (1949) indicate a maximum value which is higher than that attained in the present study. SWANSON's data were obtained at higer intensities of infrared radiation than those used for the dosage curves presented here, so that again the possibility of some general effect of intensity is suggested. Investigation of the relationship between intensity and effect over wider ranges of the dosage curve should yield information on this point. Whether or not such an effect exists has no bearing on the conclusions drawn above concerning the activated state hypothesis.

There remains another possibility which may explain the discrepancy between the maximum value obtained by SWANSON and that obtained in these studies. From the shape of the dosage curve, it is evident that there is a limit to the number of activated loci which can be acted upon by the infrared. This limit must be set by the dose of X-rays given and the spatial relations of the chromosomes. It is quite possible that there is a nonlinear X-ray dose dependence for the formation of activated states in the chromosome when the dosage differences are great enough. This is a problem which can be investigated profitably in the future. Bearing in mind these considerations, it must be emphasized that our present measure of infrared action is rather insensitive.

SUMMARY

From the data presented in this paper on the effects of infrared radiations when used as supplementary treatments with X-radiation, it has been possible to conclude the following: 1) the response to variations in dosage of infrared is nonlinear; 2) there is a definite threshold value and a definite maximum for the infrared effect; 3) a lowered intensity of infrared will cause a lower number of aberrations when the infrared is given as a posttreatment; 4) a lowered intensity of infrared has no effect on the number of aberrations recovered when the infrared is given as a pretreatment.

These facts permit a more precise distinction to be made between the various hypotheses previously advanced to explain the infrared effect. The activated state hypothesis is supported by these data.

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ALLELIC RELATIONSHIPS AND REVERSE MUTATION IN ESCHERICHIA COLI

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MANY aspects of mutation in bacteria have been studied by mutational patterns, biochemical diversity, and other phenotypic determinations without recourse to breeding test (LURIA 1947). The recombination techniques that have been developed in *Escherichia coli* K-12 (TATUM and LEDERBERG 1947) permit a more incisive analysis of the bacterial genotype. A series of lactose-negative (Lac^-) mutants in strain K-12 which revert at different rates has provided experimental material for the study of reverse mutation, the genetic factors controlling the different mutation rates, and allelic multiplicity at the Lac_1 locus.

MATERIALS AND METHODS

The intercrossable cultures used in these experiments were ultimately derived from the two auxotrophic lines of K-12: 58-161 (B^-M^-, biotinless, methionineless) and Y-10 ($T^-L^-Th^-$, threonineless, leucineless, thiamineless) in which each nutritional factor had been obtained in a discrete mutational step (TATUM 1945; TATUM and LEDERBERG 1947). Liquid cultures were grown in Difco penassay broth, while stocks were maintained on nutrient agar slants. The plating media used consisted primarily of EMB (a complete eosin-methylene blue agar) and EMS (a minimal modification of EMB), containing one percent sugar. The media were prepared from dry mixes to which water, the appropriate sugar, and other supplements were added before sterilization in the autoclave. On EMB and EMS agar fermenting colonies are opaque purple while nonfermenters are lightly tinted. Thiamine was often added to the minimal crossing media to relax selection for Th^+ (see LEDERBERG 1947). Details of media and various methods are given elsewhere (LEDERBERG 1950a; LEDERBERG *et al.* 1951). Crosses were usually carried out on EMS lactose agar for the direct scoring of fermentative characters by inspection of the colonies. After 48 hours incubation, the prototroph progeny were picked and streaked on EMB lactose to provide purified colonies for further tests.

A 125 watt Hanovia high pressure mercury vapor arc emitting a variety of wave lengths was used for ultraviolet exposures because of its high intensity output. Most of the irradiations were made directly on EMB lactose agar plates prespread with a drop of a 20 hour broth culture containing about

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10^9 cells per ml. Each plate was exposed for 7–10 seconds at a distance of 15 cm, resulting in approximately six decades of killing. The irradiations were conducted in this fashion for the empirical purpose of securing a variety of fermentation mutants.

In earlier experiments many lactose-negative mutants were intercrossed. It soon became apparent that a number of distinct loci were implicated among the different mutants which shared the lactose-negative phenotype (LEDERBERG 1948a). The most common locus was designated Lac_1^- and was selected for the present study. The principal stocks are summarized in table 1. The

TABLE 1

List of stocks.

T⁻L⁻Th⁻	Genotype	Phenotype on lactose	Source	Agent
Y-10	Lac^+	wild type	679-680	X-ray
Y-53	Lac_1^-	semi-mutable	Y-10	U-V on lactose agar
Y-70	Lac_1^-	stable	Y-53	U-V on lactose agar
W-112	Lac_1^-	stable	Y-10	U-V on lactose agar
W-133	Lac_7^-	mutable	Y-10	U-V on lactose agar
W-716		Lac^+ unstable	Y-70	spontaneous
W-844	Lac^-	mutable	W-716	spontaneous
W-888	Gal_1^-	Lac slow	W-750 × W-588	segregation from Lac^-Gal^-/Lac^+Gal^+ diploid
W-902	Gal_2^-	Lac^+	Y-10	U-V on galactose
W-1282	Lac_1^-	semi-stable	Y-53	spontaneous
B⁻M⁻				
Y-40	Lac^+V1^r	wild type	58-161	selection on T1
Y-87	Lac_1^-	mutable	Y-40	N-mustard
W-516	$Lac_1^-Pur^-$	stable	Y-87	U-V on lactose agar
W-744	$Lac_1^-Mal^{s1}$	stable	Y-87	U-V on lactose agar
W-750	$Lac_1^-Gal_1^-$	stable	Y-87	U-V on lactose agar
W-811	$Lac_1^-Gal_4^-$	stable	Y-87	U-V on lactose agar
W-842	Lac_1^-	stable	Y-87	spontaneous
W-1306	Lac^+V6^r	wild type	Y-40	selection on T6
W-1307	$Lac_1^-V6^r$	mutable	Y-87	selection on T6
W-1435	$Lac_1^-Mal^-Mtl^-V1^rV6^r$	semi-mutable	....	segregation from H-1; then selection on T6 (see Lederberg, 1949)
$B^+M^+T^+L^+$				
W-946	$Lac_1^-Gal_2^-$	semi-mutable	W-902 × Y-87	recombination

Lac_1^- designation means that preliminary tests revealed no lactose-positive recombinants in crosses of pairs of Lac_1^- mutants.

In addition to color differences of colonies on EMB or EMS lactose agar, Lac^+ and Lac^- can be distinguished in Durham tubes containing a lactose-peptone broth with bromcresol purple indicator. The change from a purple to a yellow color and the evolution of gas as end products in fermentation are observed for Lac^+ inocula. Lac^- cells give a corresponding turbid growth but the original alkaline color persists.

A test of greater precision affords a measurement of the adaptive "lactase," β-D-galactosidase (LEDERBERG 1950b). Nitrophenol is measured photometri-

cally after a short period of incubation of the chromogenic substrate, *o*-nitrophenyl β-D-galactoside with washed cells. The capacity to produce an adaptive lactase after growth in lactose broth has been lost in *Lac*$^-$ mutants. However, a small residual activity is characteristic of *Lac*$_1$$^-$ cultures. The lactose analog, *n*-butyl-β-D-galactoside, can be fermented by this group of mutants and can evoke the adaptive formation of galactosidase in amounts comparable to that formed by *Lac*$^+$. It may be noted that this substrate appears to select for

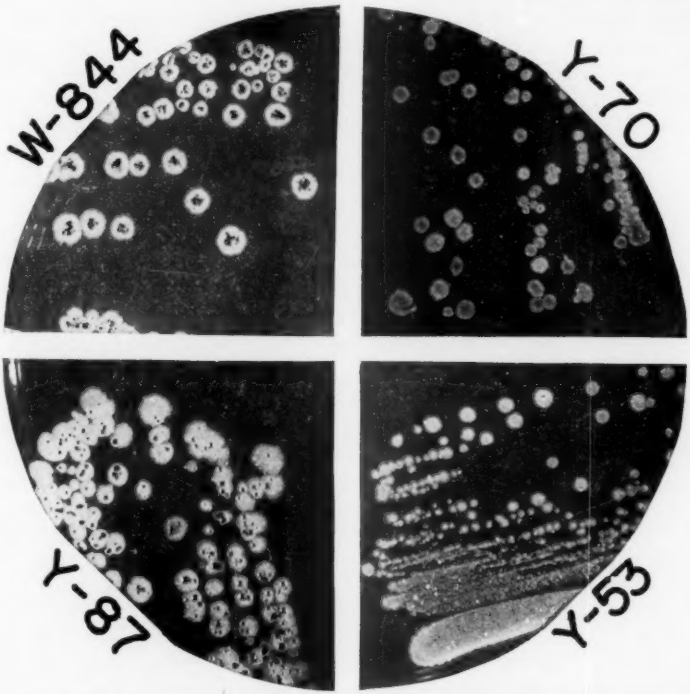

FIGURE 1.—Papillating colonies from EMB lactose agar streak plates.

spontaneous lactase-negative mutants, presumably by virtue of the toxicity of enzymatically released butanol.

PAPILLAE AND REVERSE MUTATION

Several *Lac*$^-$ strains develop outgrowths or papillae within the colonies, or marginal sectors, of the typical *Lac*$^+$ color on EMB lactose agar (figure 1). Stable *Lac*$^+$ cultures indistinguishable from type are obtained from these papillae. Such papillate forms (" *Bacterium coli mutabile* ") provided one of the earliest recognized and most cited examples of mutation in bacteria (MASSINI 1907; HADDOW 1937; MONOD and AUDUREAU 1946; LURIA 1947).

The Lac^+ papillae may represent reverse mutation to the wild type allele or mutation at another "suppressor" or "mimic" locus. To discriminate between these alternatives it is necessary to isolate the reversions and cross them with a wild type Lac^+ strain. If the Lac^+ derived from papillae are mimics of wild type, the progeny will include Lac^- recombinants, their number depending upon the closeness of the linkage between the Lac_1 and the mimic locus.

Accordingly, a single papilla was fished from each of 21 colonies of the mutable strain, Y-87 ($Lac_1{}^{-m}$). Each of the secondary colonies was carefully purified by successive streakings to rid them of adherent Lac^- cells, and five more successive single colony transfers were made after homogeneity was established. Each reversion was crossed several times with the appropriate Lac^+ (Y-10) on EMS lactose thiamine agar until about 1,000 recombinants

TABLE 2

Papillation frequencies for $Lac_1{}^-$ mutants.

Strain	No. of colonies	Mean No. of papillae	Chi-square* and DF	Probability
W-1282	100	0.61**	0.05_1	.8
Y-53	151	1.1	3.39_2	.18
	95	2.4	3.33_4	.51
	100	2.4**	10.4_4	.04
	97	3.0	3.18_3	.68
	289	4.0	18.47_5	.002
	125	4.4	3.22_4	.90
Y-87	109	4.0	11.45_6	.76
	204	6.1	5.49_6	.70
	100	14.0**	....	....

*For goodness of fit to a theoretical Poisson distribution of the same mean with n−2 degrees of freedom.
**Same experiment.

had been examined. Eventually, a total of 31,523 recombinants had been observed. One stable Lac^- exception was found in the cross Y-87R5 × Y-10. The cross was repeated but no other Lac^- appeared, and the exception might well have been a spontaneous mutation. In view of the infrequency of Lac^- among these prototrophs, the change of Lac^- to Lac^+ in Y-87 most probably constitutes a reverse mutation at the Lac_1 locus. Similar isolations of papillae were made from single colonies of Y-53 but the genetic tests were not as exhaustive. None of the 4,784 recombinants of the derived Lac^+ with Y-40 was distinguishable from wild type.

In contrast to the mutable Lac^- strains, no reverse mutants have been demonstrated for the more stable strains, even after prolonged selection. Twenty derivatives isolated from old cultures of W-112 ($Lac_1{}^{-st}$) on lactose agar or broth were all slow lactose fermenters (Lac^{sl}). Three classes of prototrophs were recovered in crosses of Lac^{sl} with Lac^+ (Y-40): Lac^+, Lac^{sl}, and Lac^{-st}. The Lac^{sl} types are therefore not reversions of Lac_1 but partial

mimics or suppressors. During this study several other suppressors of Lac_1^- mutants encompassing a wide range of fermentative ability were discovered.

The absence of mimic mutations among the mutable strains Y-87 and Y-53 is only apparent. The frequent and early production of reverse mutants in Y-87 and Y-53 would exclude the development and detection of the less frequent suppressors, especially when the reversions ferment lactose more vigorously. Where reverse mutation does not occur, the selection of Lac^{sl} mimics is facilitated.

On two separate occasions a colony of Y-70 and W-112 yielded a single normal Lac^+ papilla at the 48 hour stage. One of these carried a coincidental mutation for parathiotrophy. This culture, W-716, proved to be an extremely unstable Lac^+. When maintained on nutrient agar stock slants, it gave rise to a variety of Lac^- and Lac^{sl} types. Lactose agar delayed but did not prevent the accumulation of Lac^- cells. The Lac^- colonies themselves were unstable, including a wider divergence of distinct patterns than had previously been encountered. Of 1300 colonies examined in one derived culture, W-719, five contained a single papilla representing probably the lowest perceptible mutation rate among these cultures. The colonies of a second derivative, W-844 (figure 1), contained an average of well over 25 papillae so that counts were unreliable. The Lac^+ papillae isolated from any of these forms resembled W-716 both in their instability and the constellation of Lac^- subtypes.

The genetic position of W-716 has not yet been satisfactorily settled due to the extreme difficulty in separating mutant from recombinant Lac^-. The standard test for reverse mutation was carried out with the *V6* marker (resistance to phage T6, closely linked to Lac_1, LEDERBERG 1947) in the wild type tester (W-1306) to aid in the identification of recombinant prototrophs. About 2 percent of the latter were Lac^-. The stable Lac^+ recombinants were $V6^r$; the unstable $V6^s$, thus showing a close correlation with the parental coupling. Five single colonies of both these types were transferred to nutrient agar slants and sampled about a year and a half later. Lac^-/Lac^+ mixtures were found only in the populations classified as $V6^s$. This linkage locates the unstable mutant W-716 at or near the Lac_1 locus whose expression is modified.

MUTABILITY PATTERNS

The rate of formation of lactose-positive papillae has so far been the most characteristic index of subdivision among these otherwise phenotypically indistinguishable cultures. Representative examples of four established categories are, in order of revertibility: Y-70 and W-112, W-1282, Y-53, and Y-87 (figure 1). The frequency distribution of Lac^+ papillae was studied in this group of strains. Platings from appropriate dilutions of either 24-hour colonies on agar or broth cultures were made so as to give no more than 15 evenly distributed Lac^- colonies on each plate. Lac^+ papillae began to appear after 36–48 hours of incubation and continued to emerge as the colonies aged. The classification of colonies with 0, 1, 2, ... n papillae was recorded at the 48

TABLE 3

Test for recombination of mutability: Lac$^+$ × Lac$^-$

Cross	Lac$^+$ × Lac^{-m}	Lac$^+$ × Lac^{-st}	
	Y-10 × Y-87	Y-40 × W-112	Y-40 × Y-70
Total number of prototrophs	3,188	1,097	3,905
Percent Lac$^-$	23.2	61.3	71.3
Number Lac$^-$ tested for papillation	551	305	105
Percent Lac$^-$ stable	0	100	100

hour stage. Under these conditions, the observed numbers of papillae per colony often showed a good fit to a theoretical Poisson distribution with the same mean (table 3, figure 2). The data are consistent with the hypothesis of equally probable and independent mutations in every mutable colony.

Deviations from the theoretical distribution were also observed, especially after 48 hours. An individual strain also showed fluctuation about the mean

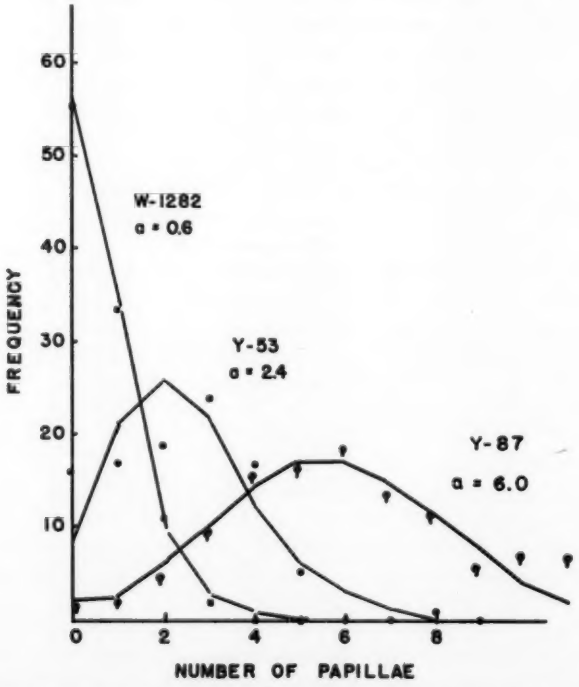

FIGURE 2.—Theoretical frequency distribution of papillae expressed as numbers of colonies with 0, 1, 2, . . . n Lac$^+$mutants for the observed mean in mutable Lac$^-$ strains. The points represent the observed values.

from experiment to experiment with occasional wide discrepancies from the Poisson distribution. These disparities probably reflect uncontrollable variations in local environment, and correlations between changes in growth rates of the *Lac*$^-$ mother cells, the mutation rate, and the capacity of the mutants to be manifested as papillae. Nevertheless, each strain retained its relative position in the mutability series.

Intercrosses were made between mutable × stable and mutable × semistable to ascertain whether a clear-cut genetic difference was involved. Among the 220 *Lac*$^-$ prototrophs from *B*$^-$*M*$^-$*Lac*$^-$ mutable (Y-87) × *T*$^-$*L*$^-$*Lac*$^-$ stable (W-112), there were only two sharply defined classes of segregants, resembling the papillation patterns of the parents. Sixty-six percent were stable, which is in fair agreement with the proportions expected from the established linkages of the nutritional markers and *Lac*$_1$. Crosses of the intermediate

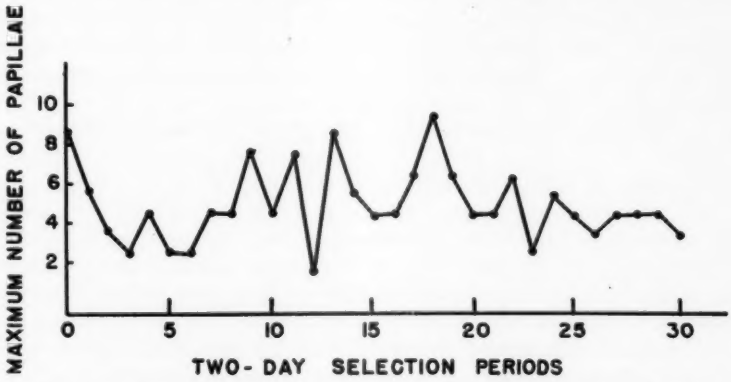

FIGURE 3.—Fluctuations in the maximum number of papillae per colony of Y-53.

alleles, Y-53 or W-1282 × Y-87 types, yield results more difficult to interpret. Individual nonpapillate prototroph colonies might represent either a genetically stable recombinant or the extreme class of a random distribution. Within this limitation, the recombinants all concorded with one or the other parent.

Spontaneous changes in mutability patterns have all led to a reduction rather than enhancement of papillation. Stable, nonpapillate colonies have been derived from Y-53 and Y-87. W-1282 of spontaneous origin occupies a mutability level intermediate between the parental Y-53 and the completely stable types. In an attempt to augment the mutability of Y-53 by recurrent selection, serial platings were made using the colony with the most papillae among 50–200 colonies at each two day selection interval. This selection was extended over thirty such stages, but no consistent trend in mutability was observed. Figure 3 illustrates the stability of the papillation pattern of this strain.

Crosses of the various *Lac*$^-$ with *Lac*$^+$ were carried out to establish whether the strains constitute a series of multiple alleles at the *Lac*$_1$ locus or

whether a mutability modifier is involved. The latter hypothesis predicts the occurrence of both stable and mutable recombinants among the *Lac*$^-$ progeny. Because only parental types have been recovered in such crosses, the argument that the differences in mutability reside within the locus is favored (table 3).

Before the conclusion can be drawn that the alleles differ in their intrinsic revertibility one further possibility must be considered. *Lac*$^+$ reversions may occur at a constant rate but the differences in papillation reflect differences in the selection and expression of *Lac*$^+$ mutants. This interpretation may be tested by determining whether large populations of *Lac*$^{-st}$ mutants are able to suppress the appearance of small numbers of *Lac*$^+$ cells when inoculated together in lactose medium. In nutrient broth where there is no obvious basis for selective differences, the growth rates of *Lac*$^{-st}$ and *Lac*$^+$ are indistinguishable. Six lactose indicator broth tubes were inoculated with a mixture of approximately 2 *Lac*$^+$ and 3×10^8 *Lac*$^{-st}$ (W-842) cells. Each tube turned yellow after 48 hours as a result of the development of a lactose-fermenting population. *Lac*$^+$ cells therefore compete effectively against preponderant populations of *Lac*$^{-st}$ in media favoring the growth of *Lac*$^+$. Similar conclusions were reached by KRISTENSEN (1944) in a more elaborate study.

INDUCED ALTERATIONS OF MUTABILITY

The mutability of a gene may be controlled by other loci (RHOADES 1941). To discover genic regulators of mutation rate, large populations of *Lac*$_1^-$ cells were subjected to ultraviolet irradiation. The surviving colonies were inspected for deviations in papillation pattern. Mutable strains were examined for non-papillating whole colonies or sectors, and similarly, *Lac*$^+$ sectors or papillae were looked for among stable strains. The suspected mutants were isolated, purified, and checked for the genetic markers of the parent strain.

Systematic study of over 33,000 survivors from a series of irradiations of the stable strains (Y-70, W-112, and W-842) yielded no examples of either mutable colonies or reverse mutants. On the other hand, stable substrains were obtained from treated populations of the mutable Y-53 and Y-87. The induced stable strains of Y-87 were studied in greater detail. Outcrosses with *Lac*$^+$ (Y-10) separated the new strains into two groups: one involving allelic or pseudoallelic changes in mutability (I) and the other consisting of forms in which a second locus was involved (II). The basis of the classification depended upon whether only parental types of prototrophs were recovered (I) or whether *Lac*$^-$ mutable colonies also appeared among the progeny (II).

The majority of the mutants belonged to the first category. They were further characterized by the retention of fermentative activity on butyl galactoside and by stability on lactose throughout the manipulations to be discussed below. Each member of group II (*Lac*$_1^-X^-$) failed to ferment butyl galactoside. The capacity for reverse mutation could be restored by replacing the modifier with *X*$^+$ by recombination or mutation.

In most of the derived stable forms of this group, the "*X*"-modifier exhibited its own phenotypic effects that facilitated its characterization. Alternatively, the modifier could be extracted in combination with *Lac*$^+$. The resynthesis of *Lac*$^{-m}X^-$, detected as stable recombinants in progeny tests of possible *Lac*$^+X^- \times Lac^{-m}X^+$ identify the extracted modifier. In order to accomplish the repeated crossings, advantage was taken of auxotrophic segregants from persistent diploids (LEDERBERG 1949).

One type of mutability modifier was associated with a nutritional requirement for purine plus thiamine in W-516 (*Pur*$^-$). The components of this combination were not separable by reverse mutation. Papillation was restored in *Pur*$^+$ reversions. The growth of *Pur*$^-$ colonies was restricted on EMB medium which is deficient in nucleic acid components. Moreover, glucose itself is less rapidly fermented by W-516 than Y-87 grown on such media. A study of the relationship of *Lac*$_1{}^{-m}$ and *Pur*$^-$ disclosed that the effects on mutability were indirect. The *Lac*$^+$*Pur*$^-$ recombinants obtained in crosses of Y-10 × W-516 were fermentatively weak. The inability of a *Lac*$^+$*Pur*$^-$ genotype to express itself normally is a clue to the absence of *Lac*$^+$ papillae in W-516 colonies. It is reasonable to conclude that the *Pur*$^-$ gene depresses glycolysis, consequently removing the selective advantage of *Lac*$^+$ mutants as confirmed in reconstructed mixtures of *Lac*$^+$*Pur*$^-$ and *Lac*$^-$*Pur*$^-$. There is no evidence that the mutation rate itself has been altered by the introduction of *Pur*$^-$ into Y-87. The *Pur*$^-$ mutation has been noticed in many subsequent irradiations of *Lac*$^+$ and *Lac*$^-$ as a small, thin colony. A similar situation was found in W-744, where the second mutation showed reduced fermentation of maltose, mannitol, and galactose.

The coincidence of a *Gal*$^-$ mutation in a *Lac*$^-$ stable strain derived from Y-87 was first recognized in W-750. Verified *Gal*$^+$ reversions were recovered as papillae on EMB galactose agar after several days of incubation. These *Lac*$^-$*Gal*$^+$ derivatives again produced papillate colonies on lactose, showing that the replacement of *Gal*$^+$ for *Gal*$^-$ restored the mutability of *Lac*$^-$ to *Lac*$^+$. The *Lac*$^+$ papillae were isolated and confirmed as back-mutants of *Lac*$_1{}^-$. Other galactose fermenters were found which were stable on lactose. Backcrosses to wild type proved these exceptions to be mutations at mimic loci rather than reverse mutants of *Gal*$^-$. Alleles at this locus which did not interact with *Lac*$_1{}^-$ were not found.

All four combinations of *Lac*$^{+/-}$ and *Gal*$^{+/-}$ were recovered from the cross W-750 × Y-10. Among 41 *Lac*$^-$ all the *Gal*$^-$ were stable, while *Gal*$^+$ papillated on lactose. Preliminary linkage tests place this *Gal* locus between (*BM*) and *Lac*$_1$. The *Gal*$^-$ mutation was extracted from the double mutant by crossing W-750 with a suitable *Het* stock (LEDERBERG 1949). Auxotrophic *Lac*$^+$*Gal*$^-$ segregants for use in crosses were obtained via a persistent diploid. In the cross Y-87 × W-888 (*Lac*$^-$*Gal*$^+ \times Lac^+$*Gal*$^-$), which may be contrasted with W-750 × Y-10 (*Lac*$^-$*Gal*$^- \times Lac^+$*Gal*$^+$), stable *Lac*$^-$ reappeared as *Gal*$^-$*Lac*$^-$ recombinants. Reversion to *Gal*$^+$ restored the revertibility of

these prototrophs on lactose agar. The same types appeared in crosses of Y-53 × *Lac*$^+$*Gal*$^-$.

The *Gal*$^-$ might influence the mutation rate of genetically susceptible alleles at the *Lac*$_1$ locus or it might affect the ability of *Lac*$^+$ mutants to express themselves as visible clones in the mother colony. The recombination analysis cannot decide between these alternatives. The second interpretation, however, is compatible with physiological tests of the genotypes. The *Gal*$^-$ allele slightly modified the appearance of *Lac*$^-$ toward *Lac*$^+$ and the *Lac*$^+$ somewhat toward slow fermentation. Galactosidase assays were made on cells of various genotypes grown in a lactose-peptone broth. The values per unit dry weight, expressed in relation to wild type K-12 as 100 percent are: Y-87 (*Lac*$_1$$^-$*Gal*$^+$) 3 percent; W-750 (*Lac*$_1$$^-$*Gal*$_1$$^-$) 20 percent; W-946 (*Lac*$_1$$^-$*Gal*$_2$$^-$) 18 percent; W-811 (*Lac*$_1$$^-$*Gal*$_4$$^-$) 25 percent; W-888 (*Lac*$^+$*Gal*$_1$$^-$) variable, 50–100 percent; W-902 (*Lac*$^+$*Gal*$_2$$^-$) 90 percent. The galactosidase assays of the *Lac*$^-$ combinations correspond reasonably well with their appearance on EMB lactose agar. The *Lac*$^+$*Gal*$^-$ types, however, appear much weaker than these assays would indicate. A loss of efficiency of lactose fermentation due to the nonfermentability of the galactose residue may explain why less acid is produced from lactose in lactose broth indicator tubes, despite the retention of lactase activity. The accumulation of monosaccharide during the fermentation of lactose by *Lac*$^+$*Gal*$^-$ but not *Lac*$^+$*Gal*$^+$ cells has been demonstrated by Barfoed's monose assay method (J. LEDERBERG, unpublished).

These results suggest that the competitive advantage on lactose of *Lac*$^+$ over *Lac*$^-$ is modified in *Gal*$^-$ genotypes. This possibility was explored by reconstitution of the selective conditions under which *Lac*$^+$ papillae were observed. *Lac*$^+$*Gal*$^+$ introduced into lactose broth, although outnumbered 10 to 100 million-fold by *Lac*$^-$*Gal*$^+$ cells, were detected after 24–48 hours, showing intense selection for *Lac*$^+$. Comparable inoculations with *Gal*$^-$ strains failed to demonstrate any preponderance of *Lac*$^+$ within that period unless they had been present initially in a ratio of 1 *Lac*$^+$: 1000 *Lac*$^-$.

The specificity of *Gal*$^-$ genes on apparent mutability of *Lac*$^-$ was studied with several nonallelic *Gal*$^-$ mutants. One of these was obtained originally as a semistable colony from ultraviolet irradiation of Y-87. This *Gal*$_2$$^-$*Lac*$^-$ had a papillation pattern resembling Y-53. *Gal*$_4$$^-$ and *Gal*$_5$$^-$ gave stable *Lac*$^-$ recombinants similar to *Gal*$_1$$^-$ when crossed with Y-87. *Gal*$_1$$^-$ also suppressed the apparent mutability of a papillate stock carrying a mutation at another *Lac* locus (*Lac*$_7$$^-$).

Combinations of *Lac*$_1$$^-$*Gal*$_1$$^-$ or *Lac*$_1$$^-$*Gal*$_2$$^-$ strains show a characteristic interaction with *Lac*$_1$$^-$ on EMB lactose agar. A stronger fermentation reaction occurred at the junction of cross-streaked inocula after 1–2 days incubation. Subculture from the lactose-positive area gave only the original *Lac*$^-$ types, and a new synergistic reaction at the juxtaposition of similar colonies. The *Lac*$_1$$^-$ mutant has already been diagnosed as producing lactase in response to butyl galactoside but not to lactose itself (LEDERBERG 1951). The influence

of the *Gal*$^-$ mutation and the synergism of *Gal*$^-$ with *Lac*$_1$$^-$*Gal*$^+$ mutants reinforce the conclusion that the *Lac*$_1$$^-$ mutants retain a considerable lactase-forming competence not ordinarily realized in the presence of lactose.

The disposition of modifiers that lack their own phenotypic effects is more difficult. The fact that all of the stable derivatives in this class lost the ability to respond to butyl galactoside suggests the possibility that a second *Lac* mutation, at another locus, had been introduced. Even though both mutations of a double *Lac*$^-$ mutant, *Lac*$_1$$^-$*Lac*$_x$$^-$, were potentially revertible, their combination would be stable as far as detectible reversions to *Lac*$^+$ is concerned. Reversion at one locus would be concealed by the mutant status of the second.

This suspicion was verified by the production of a *Lac*$_1$$^-$*Lac*$_7$$^-$ double mutant by recombination of two papillate single mutants. The double mutant was nonpapillate. Its composite nature was confirmed by crosses to wild type which separated and re-exposed the revertible components.

The status of the stabilized Y-87 derivatives that fall into this category remains in doubt, pending systematic tests for the identity of the presumed *Lac*$_x$$^-$ by crosses to standard *Lac*$^-$ mutants at various loci. Unfortunately, not all of the stocks needed for this enterprise have yet been developed in suitable form. Nevertheless, the modification of response to butyl galactoside as well as papillation of these derivatives argues against the participation of a true mutability modifier similar to *Dotted* in maize.

PSEUDOALLELISM AT THE *Lac*$_1$ LOCUS

Evidence for separating a group of four types of *Lac*$_1$$^-$ strains on the basis of their intrinsic mutation rate to a fifth allele, *Lac*$^+$ has been offered. The observed effects appeared to reside in that locus alone. However, the identification of mutant factors as alleles is at best a negative inference, derived from the failure to detect nonparental classes in a large series of tests. The scope of such experiments is subject to technical limitations inherent in the material handled (cf. STADLER 1951) and the spontaneous mutation rate of the crucial locus. Because of the applicability of selective techniques for screening large numbers, bacteria have special advantages for such studies.

In an extension of the preliminary allelism tests already cited, the crosses of *Lac*$^-$ mutants were repeated on a large scale. The presumed *Lac*$_1$ locus has been dissected into at least two components. Whereas no *Lac*$^+$ recombinants were observed among 60,000 prototrophs from Y-53 × Y-87, about 1 in 1500 from Y-87 × W-112 was lactose-positive. To avoid confusion with spontaneous reversions in the *Lac*$^-$ mutable parent, certain precautions were taken: the *Lac*$^-$ parents were freshly reisolated from a nonlactose medium to minimize the accumulation of *Lac*$^+$ mutants.

Because of its close linkage to *Lac*$_1$ (LEDERBERG 1947), *V6* served as a useful marker. Of 14 *Lac*$^+$ prototrophs from a total of 25,000 observed in a cross of W-1307 (*B*$^-$*M*$^-$*V6*^{r}*Lac*$^{-m}$ × *T*$^-$*L*$^-$*V6*^{s}*Lac*$^{-st}$) 11 were *V6*^{s} like the *Lac*$^-$ stable parent. In a parallel control experiment, of a reverted W-1307 × W-112,

all of the 24 Lac^+ prototrophs tested were $V6^r$. It may be concluded that the exceptional lactose-positive prototrophs originated by recombination rather than spontaneous mutation. Y-87 and Y-53 may therefore be considered as Lac_{1a}^- and W-112 as Lac_{1b}^-. The sequence *BM ... V6 ... a ... b ... TL* is indicated by these data. For further critical study, a second marker just to the right of $Lac_{1a,b}$ would be very useful, but has not yet been found.

Studies on heterozygous compounds of Lac_{1a} and Lac_{1b} suggest a very close relationship between these components. W-112 ($V6^s a^+b^- V1^s Mtl^+$) was crossed on EMS mannitol agar with a filial Y-53 derivative, W-1435 ($V6^r a^- b^+ V1^r Mtl^- Het$). *Het* is a factor that promotes nondisjunction (LEDERBERG 1949). Heterozygous diploid prototrophs were isolated on the basis of their segregation for *Mtl*, *V1* and *V6*. Such diploids are presumably a^+b^-/a^-b^+ at the Lac_1 locus. Although they showed little or no fermentation of lactose, this constitution was verified by the segregation of Lac^- stable ($V6^s a^+b^-$) and mutable ($V6^r a^-b^+$) haploids. Crossing over to yield a^+b^+ occurs relatively frequently and results in marked papillation. Heterozygotes which are almost certainly a^+b^+/a^-b^- occur among exceptional lactose-positive prototrophs from the same cross. In distinction to the opposed "repulsion" heterozygotes, these "coupling" diploids segregate lactose-positive and stable lactose-negative haploids. The latter are assumed to be a^-b^-, complementary to the a^+b^+ crossovers. Because the presumed a^+b^+/a^-b^- is lactose-positive while a^+b^-/a^-b^+ is lactose-negative, a "position effect" is suggested.

A group of mutants has been designated as Lac_4^-. These mutants differ from Lac_1^- in showing little or no residual lactase when grown on butyl galactoside, and when crossed with Lac_1^- give lactose-positive recombinants with a frequency of 0.1 to 0.2 percent (LEDERBERG 1951). Crosses of Lac_4^- with W-112 (a^+b^-) or Y-53 (a^-b^+) have given identical results. In addition to the rare Lac^+ crossovers, lactose-positive nondisjunctional exceptions were found (LEDERBERG 1949). In contrast to compounds of a^-b^+/a^+b^-, heterozygotes of the constitution $Lac_4^-a^+b^+/Lac_4^+a^-b^+$ and $Lac_4^-a^+b^+/Lac_4^+a^+b^-$ approach Lac^+ in their lactase activity. In spite of the close linkage of Lac_1 with Lac_4, these loci appear to function independently of each other and are therefore assigned different symbols.

DISCUSSION

Multiple alleles can be characterized by differences in mutability as well as in physiological expression. For example, two inositol-dependent mutants have been described in Neurospora that represent allelic forms indistinguishable from one another except for their capacity to revert to wild type upon suitable mutagenic treatment (GILES 1948). The mutability difference segregated in the ascospore progeny when the two mutants were crossed.

Recurrent mutants with different rates of spontaneous reversion were identified at the Lac_1 locus in *E. coli*. Qualitative examination disclosed four

categories. Additional intergrading alleles were suspected but may be unclassifiable by ordinary means. The number of possible configurations at this locus may be very large, although empirical tests for precise separation of all the potential alleles have not been formulated. RHOADES (1941) has already proposed the concept of a continuous spectrum of mutation frequency of which only a few points may be recognized.

Recent genetic research has revealed the complexity of several genes. The components of the *R* (STADLER 1951) and the *A* (LAUGHNAN 1948) alleles in maize have been separated by their mutability and physiological action where recombination tests at first failed to subdivide the locus. Intensive studies of large numbers of progeny among allelic recurrences in *E. coli* (LEDERBERG 1951), Neurospora (BONNER 1951; GILES 1951), and Drosophila (GREEN and GREEN 1949; LEWIS 1951; TAKU-KOMAI 1950) have also revealed a fine structure in the loci involved.

In *E. coli* spontaneous or induced changes in mutability pattern at the Lac_1 complex invariably involved alterations to more stable types. The miniature alleles in *Drosophila virilis* which differ in their mutability, however, are able to mutate to either more stable or unstable forms (DEMEREC 1941). A similar situation exists for an unstable Mal^- mutant in *E. coli* (LEDERBERG *et al.* 1951).

The intrinsic factors determining the degree of mutability are often autonomous, i.e., appear to reside within the locus. Other genotypic influences have been discovered, however. The existence of at least two mutability modifiers of the *R* locus has also been recently suspected (STADLER 1949). The most clear-cut specific intergenic regulator of mutability has been described by RHOADES in Dotted maize (RHOADES 1938, 1941, 1945).

Technical difficulties have precluded a biochemical analysis of the mutability interactions in maize. On the other hand, bacteria would be eminently suitable for exploring such effects. Modifiers that suppressed the formation of Lac^+ reversions in unstable Lac^- strains were readily found. Closer examination revealed that the stable Lac^- types had remained potentially mutable, but the mutants could not be expressed in combination with certain alleles at other loci. In strains W-516, W-744, and W-750, a reduced selective advantage interfered with the expression of Lac^+ mutants. In addition, Gal^- modified the phenotype of the Lac^- cells by enhancing galactosidase activity. These genetic factors may be summarized as affecting the conditions under which the Lac^+ mutants are favored. No gene-induced alteration of the intrinsic mutation rate of another gene was discovered in this study.

As far as could be determined, the Lac^+ derivatives of each mutable Lac^- strain were reverse mutants. Suppressor loci were found, however, by selecting lactose fermenters from stable strains. These loci are latent in the wild type Lac^+. They can be detected as mutations which mimic wild type by suppressing the effect of Lac_1^- mutation.

The two suppressors reported in Neurospora are each less efficient than the

comparable reversions (HOULAHAN and MITCHELL 1947; GILES 1950). This was also true for several Lac_1^+ mimic factors. The Lac^+ of W-716 which most closely resembled Lac_1^+ was rapidly eliminated in a lactose-free medium. The inefficiency of suppressor mutants may perhaps be ascribed to either genic inefficiency or to imbalance with the rest of the genotype.

Duplication of a locus has been postulated as one mechanism for the origin of suppressors and pseudoalleles (SCHULTZ and BRIDGES 1932; HOULAHAN and MITCHELL 1947; STEPHENS 1948). One may visualize the transfer of such a duplication to another location, followed by gradual modification of its original activity and eventual evolutionary divergence both in microorganisms (LEDERBERG 1948b) and in higher forms (STEPHENS 1951).

SUMMARY

A series of recurrent Lac_1^- mutants was classifiable by the rates of reversion to Lac^+. Four major types were recognized on the basis of the average number of Lac^+ papillae per colony under standard conditions. All alterations of mutability, whether spontaneous or induced, were in the direction of greater stability. The papillae isolated from the mutable Lac^- strains were back-mutations as proven by backcrosses to wild type. However, stable strains gave rise to mimic reversions (suppressors) phenotypically less competent than wild type.

The primary differences in mutability were inseparable from the locus in recombination experiments. Other loci controlling apparent mutability were implicated in secondary stable types isolated from mutable strains. Further studies showed that the second mutation lowered the selective advantage of potential Lac^+ mutants but did not necessarily modify the intrinsic mutability directly.

The subdivision of the Lac_1^- locus into two components, *a* and *b*, was revealed by the rare occurrence of Lac^+ recombinants among intercross progeny. a^-b^+/a^+b^- heterozygotes were phenotypically lactose-negative. On the other hand, the a^+b^+ crossovers, both in haploids and in heterozygotes were phenotypically lactose-positive, indicating a "position effect."

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LONG TERM ADAPTATION TO GALACTOSE BY YEAST[1]

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RECENT revival of interest in the problem of substrate influenced modification of segregation ratios involving "slow" fermentation of galactose has resulted in conflicting interpretations of the phenomenon of long-term adaptation. WINGE and ROBERTS (1948) attributed the delay in gas production by haploids to the gradual accumulation of galactozymase, in the presence of substrate, by "long-term" adapting clones. They held that increments in enzyme concentration occurred uniformly in every cell to yield a population which, after 6–7 days of contact with galactose, produced gas at a rate characteristic of genetically dominant (G) rapid fermenters of galactose. Subsequent transfers of such adapted cultures to galactose medium exhibited rapid fermentation within 1–2 days much as any segregant carrying the gene for rapid fermentation. However, culturing of adapted segregants for variable periods on galactose-free substrate yielded, with notable exceptions, populations which reverted to the original slowness in fermenting galactose. To explain this behavior the presence of a gene g_s (allelic to G) which elicited a gradual acquisition of enzyme in the cell was invoked. In advancing this view, WINGE and ROBERTS denied the possibility of mutations of genetically negative g segregants to the rapid fermentation type to yield long-term adapting clones.

SPIEGELMAN, SUSSMAN and PINSKA (1950) likewise excluded mutations but, on the other hand, held that long-term adaptation was induced by substrate in only a small proportion of cells, this modification of ability to form enzyme being transmitted through cell generations by cytoplasmic elements which increased autocatalytically. They demonstrated that reversions to slowness, in the absence of substrate, were mass phenomena involving the overwhelming majority of adapted cells and occurred between the fifth and sixth cell generations. Furthermore, they concluded from the above fact as well as a variance analysis that mutation and selection were inconsequential agencies in effecting long-term adaptation.

MUNDKUR and LINDEGREN (1949), however, interpreted long-term adapted clones as originating from a negative g segregant, some cells of which eventually mutated to G. The adapted culture was thus a mixed population of cells carrying g or G genes and therefore a phenotype for which the symbol g_x (where x denotes the number of days elapsing before gas release in Durham tubes) was used. The view that a mutation-selection agency was operative in the adaptation is based on an extensive genetical analysis involving 159 hybrid

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asci and the fact that when large-colonied, rapid fermenter (G) variant cells recovered from a slow culture were out-crossed to negatives in the absence of galactose, half the number of haploid segregants in the hybrid asci were rapid fermenters of galactose. Furthermore, the results of $g \times g_x$ crosses were not such as could be accounted for on the basis that slow fermentation is determined by a specific gene in the stocks employed.

The experiments reported in the present paper were designed with a view to determining the basis for the divergent views on the phenomenon of long-term adaptation and show that they have been due to the use of unidentical yeast stocks by the different authors.

MATERIALS AND METHODS

Four different yeast strains have been used in these studies. Cultures 6166, 12475 and 3246 are genetically well-marked haploid members of pedigrees developed in this laboratory; and haploid culture SC 1 originating from *Saccharomyces chevalieri* (NRRL Y-1345) obtained from DR. L. J. WICKERHAM. The latter culture is identical with the one from which SPIEGELMAN *et al.* isolated a single cell derivative ($C_1{}^d$) in their investigation. All four cultures were tested individually prior to use and found to be incapable of copulating illegitimately and producing ascospores. Cultures 12475 and 3246 are good nonfermenters of galactose and do not evolve gas even on extended periods of contact with the sugar. Cultures 6166 and SC 1 are slow, the former being characterized by widely variable periods of delay before gas is released while the latter almost always produces gas on the fifth or sixth day of contact with galactose. In this respect, at least, the culture is no different from isolate $C_1{}^d$ employed by SPIEGELMAN and co-workers.

The technique of mass matings was employed for the production of diploids. Single spore isolations were made with the aid of a de Fonbrune micromanipulator; cell counts were made with a haemacytometer.

Media: Galactose (Pfanstiehl Chemical Company) was used as a carbon source in the following three media: 1) Liquid yeast extract-peptone medium: Yeast extract 1 cc, Peptone 3 gm, KH_2PO_4 2 gm, $MgSO_4$ 1 gm, Agar 30 gm, Water 1 l. 2) Burkholder's synthetic medium (1943) modified from the original formula by a reduction of metals to ¼ and of KI to ¼ the usual concentrations. 3) Wickerham's carbon-free basal medium.

Eosin-methylene blue agar when used was made according to the concentrations used by SPIEGELMAN.

EXPERIMENTAL RESULTS

Recovery of variant colonies in galactose pour-plates. The haploid slow clones employed in our previous analysis as well as culture 6166 used in the present study were derived through an extensive program of interspecific matings carried on by MRS. LINDEGREN in this laboratory involving *S. cerevisiae, S. chodati, S. microellipsoideus* and *S. carlsbergensis* undertaken with the object of incorporating new characters in our breeding stocks. Hence they

may not be justifiably comparable, in respect to slow fermentation, with cultures of *S. chevalieri*. SPIEGELMAN *et al.* (1950) attributed the low variance in colony size classes in galactose pour-plates of this species to a substrate induced cytoplasmic variation although no genetic analysis was attempted in their experiments in support of this view. Although the results of our previous genetic analysis with our stocks cannot be explained on any basis other than that of mutation and selection, no satisfactory variance study was undertaken. The 20 galactose pour-plates reported in our experiments were made with the object of detecting rapid fermenter variant cells (occurring in glucose-grown

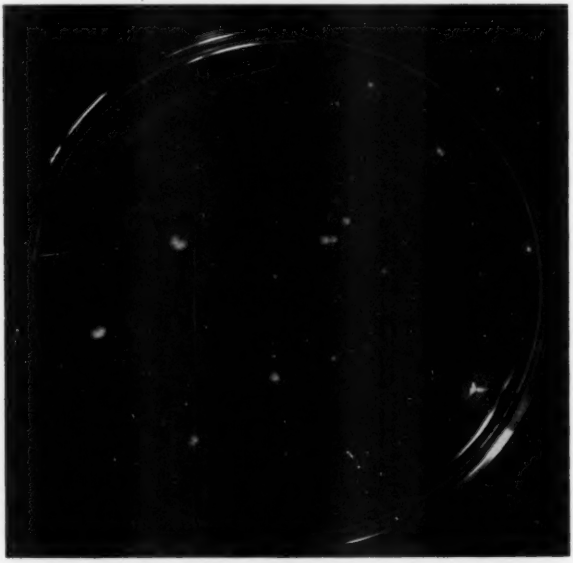


FIGURE 1.—Rapid-fermenter mutant colonies of culture 6166 in galactose pour-plate. They originate from initially non-fermenter populations grown in glucose prior to plating. The background consists of enoromus numbers of single cells or small clusters of cells that cannot ferment galactose. A layer of non-nutrient agar covers the galactose agar and alcohol produced by growth of the large colonies stimulates division of the surrounding non-fermenters of galactose.

slow populations) rather than of studying the fluctuations in variant colony numbers. A more extensive variance analysis coupled with genetic control therefore was felt essential in view of SPIEGELMAN'S findings in *S. chevalieri*.

Tables 1 and 2 record the results of plating out in Burkholder's synthetic galactose agar of culture 6166 separately from 62 glucose tubes as well as in multiple samples from the same glucose tube. In either case the entire, mature glucose-grown populations were utilized in making the pour-plates. Figure 1 shows the appearance of the variant colonies on one such pour-plate. The large, variant colonies when isolated from the plates in pure culture and transferred successively 5–6 times on glucose medium did not lose their capacity for

TABLE 1

Series of Similar Cultures

Experiment to determine variance of G colonies in galactose pour-plates made from a series of 0.5 cc or 1.0 cc glucose grown populations of 6166. Initial population seeded in each tube, 280 slow cells. Burkholder's synthetic medium. Entire mature population from each glucose tube was used in making a single pour-plate. Counts made 5th day after plating.

0.5 cc glucose tubes		1.0 cc glucose tubes	
Galactose plate	No. *G* colonies	Galactose plate	No. *G* colonies
1	9	1	11
2	6	2	7
3	11	3	12
4	13	4	17
5	9	5	12
6	9	6	13
7	8	7	9
8	12	8	11
9	11	9	15
10	17	10	12
11	13	11	8
12	10	12	7
13	13	13	12
14	9	14	4
15	4	15	11
16	7	16	13
17	11	17	10
18	4	18	9
19	7	19	20
20	13	20	14
21	5	21	11
22	7	22	6
23	11	23	12
24	14	24	11
25	9	25	8
26	8	26	19
27	10	27	11
28	13	28	11
29	10	29	19
30	10	30	6
		31	18
		32	11
$m = 9.77$		$m = 11.56$	
$\sigma = 3.05$		$\sigma = 3.94$	

rapid fermentation of galactose. Furthermore, when cells from 20 such large colonies were diploidized separately with culture 3246, two of the segregants in each of a total of 58 asci were of the expected *G* type, confirming the mutant nature of the variant cells recovered from galactose plates. The entire life cycle—copulations between haploids, germination and culture of segregants—was passed on Burkholder's synthetic galactose-free medium. The first contact of the segregants with galactose was in Durham tubes.

In spite of their failure to revert to slowness on transfer to galactose-free substrate and even on passage of the sexual cycle through galactose-free medium—a behavior inconsistent with the cytoplasmic perpetuation of the

TABLE 2

Experiment to determine variance of G colonies in samples taken from the same glucose-grown population of 6166. Eight samples from each of five 0.5 cc glucose and 1.0 cc glucose populations were made. The entire population was used in sampling. Burkholder's syntbetic medium. Initial inoculum in glucose, 280 slow cells of 6166. Counts made on 5th day after plating.

Samples from 0.5 cc glucose tubes				Samples from 1.0 cc glucose tubes			
Tube	Samples	No. colonies per gal. plate		Tube	Samples	No. colonies per gal. plate	
I	1	8	Average	I	1	11	Average
	2	9	per plate:		2	2	per plate:
	3	11	10.3		3	12	7.6
	4	11			4	10	
	5	16			5	8	
	6	7			6	5	
	7	8			7	2	
	8	13			8	11	
II	1	8	Average	II	1	5	Average
	2	6	per plate:		2	8	per plate:
	3	7	8.0		3	5	7.7
	4	6			4	10	
	5	9			5	9	
	6	12			6	9	
	7	6			7	4	
	8	13			8	12	
III	1	5	Average	III	1	5	Average
	2	5	per plate:		2	8	per plate:
	3	9	8.3		3	7	7.6
	4	8			4	7	
	5	10			5	8	
	6	4			6	10	
	7	14			7	5	
	8	12			8	11	
IV	1	5	Average	IV	1	12	Average
	2	6	per plate:		2	10	per plate:
	3	9	6.8		3	5	8.7
	4	12			4	7	
	5	6			5	12	
	6	3			6	9	
	7	8			7	6	
	8	6			8	9	
V	1	7	Average	V	1	5	Average
	2	4	per plate:		2	7	per plate:
	3	3	5.8		3	7	7.5
	4	6			4	10	
	5	5			5	9	
	6	5			6	5	
	7	9			7	8	
	8	8			8	9	

adapted state—it will be seen from tables 1 and 2 that the variance in numbers of large colonies is of a very low order. In this respect, the behavior of our stocks on plating resembles the results obtained for isolate C_1^d of *S. chevalieri*. Two significant differences must, however, be emphasized: 1) Size differences. In dense seedings (3×10^8), of 6166 and other slow clones of our stocks, the large variant colonies on galactose plates vary in diameter from 6–15 mm on the 13th or 14th day of incubation at 30°C. Microscopic examination of the plate showed single cells or occasionally cell clusters which had not grown beyond four divisions. Similar seedings of dense populations of *S. chevalieri* revealed two apparent size classes visible to the naked eye; the larger colonies in such plates were uniformly sized and approximately 1 mm in diameter. 2) Numbers of colony size classes. An average of about 11 large *G* colonies

TABLE 3

Discrepancies in periods of delay before gas release in four percent galactose Durham fermentation tubes inoculated with uniform quantities of 6166 (slow). 30 tubes of each medium.

No. of days prior to gas release	No. of tubes showing gas		
	Burkholder's	Peptone-LYE	Wickerham's
6	1	0	0
7	1	0	3
8	0	0	3
9	1	2	1
10	2	3	0
11	2	1	2
12	1	2	1
13	1	0	0
14	2	1	2
15	2	2	2
16	0	0	3
17	2	2	1
18	0	2	0
19	1	0	1
20	1	0	0
21	2	0	0
22	0	3	1
23	0	2	1
24	0	0	0
25	0	0	1
26	1	2	0
27	2	0	0
28	0	0	0
29	0	1	1
30	2	0	2
31	2	0	1
32	0	1	0
33	3	2	0
34	0	0	1
35	1	0	1
	All tubes bore gas	4 tubes did not hold gas (until medium evaporated)	2 tubes did not hold gas (until medium evaporated)

appeared in dense platings of 6166 as against an almost 50 percent proportion of the 1 mm colonies in similar heavy platings of *S. chevalieri*. Identical size effects were noted in EMB plates made with the latter species.

Figure 2 shows the result of an experiment designed to determine whether the low order of variance in numbers of mutants in densely seeded galactose pour-plates made with slow culture 6166 could have been caused by crowding

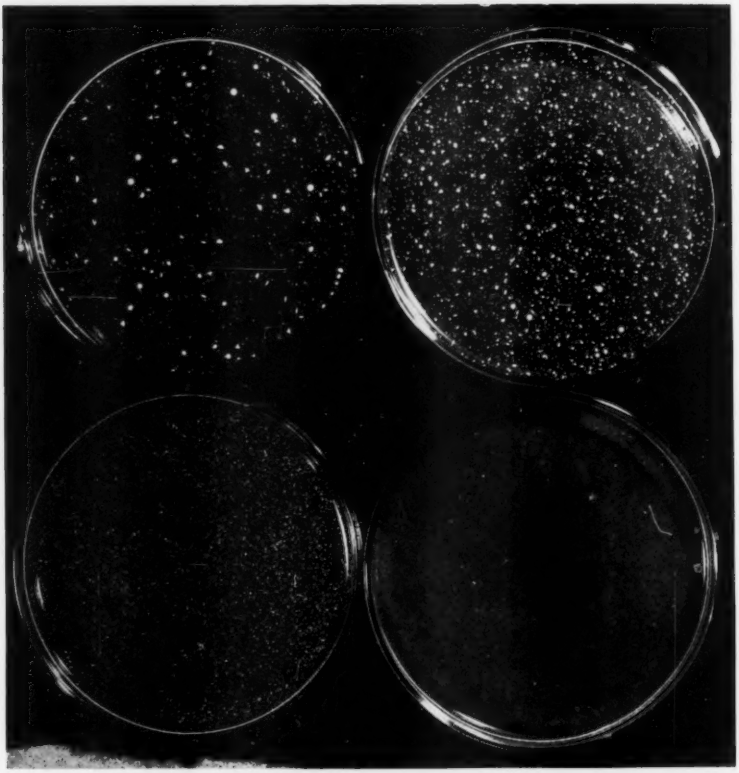


FIGURE 2.—Galactose pour-plates made at intervals from mixed populations of mutant and normal cells of 6166 previously grown in glucose.

and the possible inhibition of expression of mutant colonies. Mixtures of known numbers of mutant cells, recovered in pure culure from a galactose plate, and slow [2] cells were grown in glucose-Burkholder's liquid synthetic medium and plated out at intervals into galactose-Burkholder agar with a view to determining the frequencies of large mutant colonies. Sixty rapid fermenter

[2] This refers to glucose-grown cells and not to cells taken from a galactose fermentation tube in which gas had already registered.

cells derived from the slow culture 6166 were mixed with 2900 cells from a glucose-grown culture of the latter. These mixed populations were incubated at 30°C in uniform series of tubes with 0.5 cc glucose medium and plated out in galactose agar at five hour intervals. Pour-plates utilizing the entire populations as well as diluted samples from such glucose tubes were made. It is evident from figure 2, showing the effect of plating out entire populations, that the number of mutant colonies increases with time in spite of crowding, suggesting the absence in glucose of suppressive effects exercised by the slow cells on the relatively smaller population of mutant *G* cells with which they were initially incubated.

In addition to the genetical confirmation of the mutant nature of the large colonied variants recovered in galactose plates made with our slow stocks (MUNDKUR and LINDEGREN 1949) and in the present study, further support for this view is lent by the widespread discrepancies in delays prior to gas formation by the slow clones. These discrepancies obtained not only in segregants from asci derived from matings involving g_x parents but also when the same culture is inoculated into a series of galactose fermentation tubes. Fluctuations in the period of delay prior to active gas release when culture 6166 is inoculated into Durham tubes appear in table 3.

A uniform suspension (5×10^8 per ml) of 6166 was made in Seitz-filtered, 12 percent glucose-free galactose and 1 cc of this suspension was pipetted into each of a series of Durham tubes holding 2 cc sterile sugar-free medium, to bring the final concentration of galactose in each tube to four percent and ensuring the introduction of fairly constant populations of 6166 into each tube. The fluctuations in latent periods range from 6–35 days and are suggestive of the random nature of the adaptation even though uniform quantities of inoculum of the same culture are involved.

In contrast to this behavior of 6166, inoculations of *S. chevalieri* (SC 1) under identical conditions resulted in a remarkably simultaneous active evolution of gas on the 5th or 6th day in all tubes.

GENETIC ANALYSES

The crucial criterion for determining the nature of a variant character—its genic or cytoplasmic basis—consists in analyzing the segregants in asci derived from mating variant cells with normal cells. Genetic data reported previously as well as the more extensively analyzed Mendelian segregation of the variant character in the present study have proved that a gene is involved in long-term adapting clones of stocks developed in this laboratory. We may reasonably presume that each large variant colony of 6166 on galactose plates is of single cell origin. The fact that hybrid asci obtained by crossing cells taken from such large colonies with non-fermenter cells yield asci in which 2 : 2 segregation of rapid fermenters and non-fermenters are found in accordance with Mendelian expectation proves that the variant colony cells arose as mutants. Furthermore, in spite of continued subculture on galactose-free substrate, the mutant cultures maintained their rapid fermentative capacity.

A two to two segregation of *G* to *g* on out-crossing variant cells with *g* cells would not obtain, however, if the origin of variant cells were due to a cytoplasmic modification resulting in an ability to form galactozymase transmissible through successive cell generations by autocatalytically perpetuated cytoplasmic elements. The crucial test for such a conclusion would consist in hybridizing variant rapid fermenter cells with negative cells in the continual presence of galactose since, otherwise, according to the demonstration by SPIEGELMAN *et al.*, ability for rapid fermentation would be dissipated in the course of a few cell divisions, in the absence of contact with galactose.

Genetic analysis of such hybrids made during their continual contact with galactose should yield more than two rapid fermenter cells per ascus, since the galactozymase possessed by the derived (adapted) parent cells would be expected to be distributed evenly and stabilized by substrate in the diploid and among the four ascospores. An analysis of this sort was not attemped in the experiments of WINGE and ROBERTS or of SPIEGELMAN *et al.* though other experimental procedures led them to infer a cytoplasmic basis for the nature and perpetuation of the adapted state of *S. chevalieri.*

In view of the different behavior patterns exhibited by slow stocks of Carbondale cultures and by *S. chevalieri,* which are suggestive of differing modes of long-term adaptation, genetical analysis on the lines outlined above was felt necessary to evaluate the phenomenon in *S. chevalieri* in more detail.

In our plating exoeriments with SC 1 we have been unable consistently to correlate the larger colonies on galactose with the rapid fermentation character, in contrast to SPIEGELMAN's experience with isolate C_1^d of this species. Hence, selection of clones of this species capable of fermenting galactose within 24 hours was effected by other means. A loopful of this culture inoculated into a galactose fermentation tube produced gas after six days incubation as expected and on subsequent transfer to a new galactose tube yielded gas within 24 hours. Five similar transfers of this adapted, rapid fermenter culture were made and a sample of the adapted yeast from the final tube was carried as the stock culture on galactose medium. It may reasonably be presumed that this highly selected population of cells is fairly homogeneous and consists almost entirely of cells capable of a 24 hour fermentation, i.e., of phenotype *G*.

Matings of this adapted population with a haploid (12475) from our stocks carefully selected for its inability to ferment galactose were made under three conditions. (a) Copulations of haploids, sporulation and isolation and culture of the segregants were all effected in the presence of glucose as the sole carbon source in the medium. The first contact of the segregants with galactose occurred when they were tested for their ability to ferment galactose. (b) The entire life cycle was carried out on a medium comprising a mixture of two percent glucose and two percent galactose. Diagnosis of the segregants was made in galactose medium. (c) The entire life cycle was carried out on a medium with galactose as the sole carbon source. The medium used throughout these experiments was Burkholder's synthetic.

Since the matings involve adapted (*G*) populations and negative *g* cells as

parents, the segregants recovered in each of these three crosses could be expected to exhibit different behaviors if the adapted state were due to cytoplasmic elements dependent for their perpetuation on the presence of substrate. That is, cross (a) should yield asci showing only a segregation of two g_x and two g, since reversions would be expected in the absence of contact with galactose during the life cycle; cross (b) should yield, depending on diauxic effects (MONOD 1947), mainly $2\,g_x : 2\,g$ asci, and possibly $2\,G : 2\,g$ asci; cross (c), however, would be expected by virtue of the stabilizing influence on the galactozymase of the continually present galactose, to yield asci with $2\,G : 2\,g$ segregants.

TABLE 4

Tetrad analysis of a cross between unadapted S. chevalieri (slow) and a non-fermenter of galactose. Copulations of haploids, isolation and culture of segregants made in glucose medium. Diagnosis of segregants in glucose-free galactose medium. Note segregant 1449.

SC1 × 12475 g			
1393 g	1413 g_5	1433 g	1453 g
1394 g_3	1414 g	1434 g_6	1454 g
1395 g	1415 g_3	1435 g	1455 g_8
1396 g_9	1416 g	1436 g_4	1456 g_5
1397 g	1417 g_5	1437 g	1457 g
1398 g_9	1418 g_5	1438 g_6	1458 g_8
1399 g	1419 g	1439 g	1459 g_6
1400 g_7	1420 g	1440 g_4	1460 g
1401 g	1421 g	1441 g_{10}	1461 g_5
1402 g	1422 g	1442 g	1462 g_5
1403 g_9	1423 g_5	1443 g_{11}	1463 g
1404 g_6	1424 g_6	1444 g	1464 g
1405 g	1425 g	1445 g_5	1465 g
1406 g_3	1426 g_{13}	1446 g_5	1466 g_{11}
1407 g	1427 g	1447 g	1467 g_9
1408 g_6	1428 g_7	1448 g	1468 g
1409 g_5	1429 g_3	1449 G (28 hrs.)	
1410 g_4	1430 g	1450 g	
1411 g	1431 g_7	1451 g	
1412 g	1432 g	1452 g_6	

Unfortunately, cross (c) was not satisfactory since cell copulations were extremely poor and sporulation could not be induced. This is in accord with our general experience that cell fusions between two haploids, one of which is a non-fermenter of a sugar which occurs as the sole carbon source in the medium, are very unsatisfactory.

Table 4 shows segregations obtained in asci derived by mating unadapted SC 1 with 12475. Copulations, sporulation, and isolation and culture of the segregants were effected throughout on glucose medium. Nineteen four-spored asci were dissected and, with one exceptional ascus, 18 showed the expected segregation of two slow to two negatives. The exceptional ascus yielded two

TABLE 5

Eleven asci derived by crossing adapted S. chevalieri with a non-fermenter of galactose. Adaptation of the former parent was achieved by serial transfers through galactose medium. Copulations, isolation and culture of segregants in two percent glucose plus two percent galactose medium. Diagnosis in glucose-free galactose medium (synthetic).

SC 1 (rapid fermenter) × 12475 g		
1193 g	1209 g_3	1225 g_7
1194 g	1210 g	1226 g
1195 G (11 hrs.)	1211 G (40 hrs.)	1227 g_9
1196 G (11 hrs.)	1212 g	1228 g
1197 g	1213 g	1229 G
1198 g_5	1214 g_4	1230 g
1199 g_7	1215 G (19 hrs.)	1231 g_6
1200 g	1216 g	1232 g
1201 g	1217 G (19 hrs.)	1233 g_7
1202 g_3	1218 g	1234 g
1203 g	1219 g	1235 g
1204 g_4	1220 G (19 hrs.)	1236 G
1205 g_{15}	1221 g	
1206 g_7	1222 g_{12}	
1207 g	1223 g	
1208 g	1224 g_{10}	

TABLE 6

Fifteen asci derived by mating 1194 g with 1196 G (table 5). Matings, sporulation, isolations and stock cultures carried throughout in glucose medium. Diagnosis in glucose-free galactose medium.

1194 *g* × 1196 *G*		
1285 G (24 hrs.)	1305 G (20 hrs.)	1325 G (20 hrs.)
1286 g	1306 g	1326 G (20 hrs.)
1287 g	1307 G (20 hrs.)	1327 g
1288 G (24 hrs.)	1308 g	1328 g
1289 g_{14}	1309 g	1329 G (20 hrs.)
1290 g	1310 g	1330 g
1291 g_{11}	1311 g_6	1331 G (29 hrs.)
1292 g	1312 g_8	1332 g
1293 g_7	1313 g	1333 g
1294 g	1314 g_3	1334 G (20 hrs.)
1295 g	1315 g_5	1335 g
1296 g_9	1316 g	1336 G
1297 g	1317 g	1337 G
1298 g	1318 g	1338 g_6
1299 g_8	1319 g_6	1339 g
1300 G (19 hrs.)	1320 g_4	1340 g
1301 g_7	1321 g_8	1341 g
1302 g	1322 g	1342 G (20 hrs.)
1303 g_6	1323 g_8	1343 G (20 hrs.)
1304 g	1324 g	1344 g

negatives, one six day slow and an unusual 28-hour rapid fermenter clone (1449) which maintained the fast fermentation character even on continued culturing on glucose medium. Similar failures to deadapt fast fermenter clones by culturing on galactose-free wort medium were also encountered by WINGE and ROBERTS.

However, out of 12 asci from a cross between an adapted *S. chevalieri* (SC 1) parent and 12475 in glucose medium (cross (a) described above) 10 asci showed a Mendelian segregation of two slows and two negatives in each tetrad, while the remaining two tetrads showed a Mendelian segregation of two rapid fermenters and two negatives. This suggests that the frequency of rapid fermenters can be increased when the adapted parent is involved in a mating with 12475. Furthermore, each of the four rapid fermenter segregants recovered from this mating were incapable, like 1449 derived from the cross involving the unadapted parent, of reverting to slowness on transfer to glucose medium.

Table 5 records the results of crossing adapted *S. chevalieri* (SC 1) with 12475 in a medium comprising two percent glucose as well as two percent galactose. Adaptation for rapid fermentation of the former parent was achieved by serial transfer of a glucose grown culture through five galactose Durham tubes. Eight rapid fermenter clones appeared among a total of 44 segregants from 11 asci and, as before, failed to deadapt to slowness on testing in galactose fermentation tubes after three transfers on glucose medium. All asci analyzed were found to be true hybrids as revealed by the segregation of other marker genes. To determine the nature of the stable rapid fermenter clones obtained from this cross, fifteen four-spored asci were recovered by mating culture 1194 (*g*) with 1196 (stable *G*). These matings and isolations of ascospores were

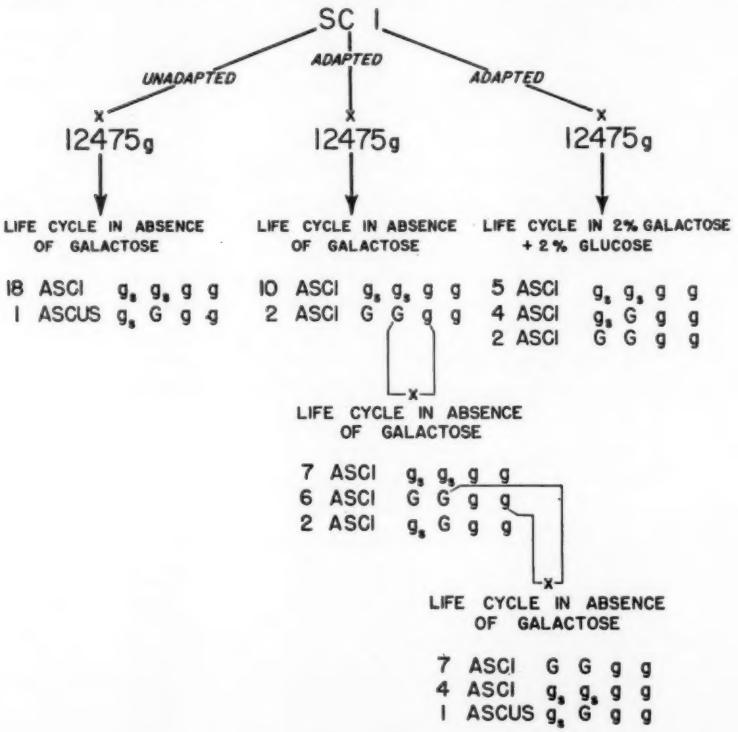
TABLE 7

Twelve asci derived by mating 1306 g with 1307 G (table 6). Matings, sporulation, isolations of spores and stock cultures carried throughout on glucose. Diagnosis in glucose-free galactose medium.

1345 G (29 hrs.)	1361 g_9	1377 g
1346 g	1362 g	1378 g_9
1347 G (29 hrs.)	1363 g_4	1379 g
1348 g	1364 g	1380 g_7
1349 G (29 hrs.)	1365 g	1381 G
1350 g	1366 G (26 hrs.)	1382 g
1351 G (29 hrs.)	1367 G (26 hrs.)	1383 G
1352 g	1368 g	1384 g
1353 g	1369 g	1385 g
1354 g	1370 g_4	1386 g
1355 g_4	1371 g	1387 G (20 hrs.)
1356 g_4	1372 G	1388 G (20 hrs.)
1357 g_6	1373 G (29 hrs.)	1389 G (20 hrs.)
1358 g_8	1374 g	1390 g
1359 g	1375 g	1391 g
1360 g	1376 G (29 hrs.)	1392 G (20 hrs.)

made in glucose medium in order to favor further reversions. Regardless of these conditions, it will be seen from table 6 that six tetrads out of 15 exhibited a segregation of two fast and two negative, two tetrads exhibited one fast fermenter in each, while seven tetrads each possessed two slow and two negative.

This phenomenon prevailed even when a similar cross in glucose between two segregants from the same ascus (1306 $g \times$ 1307 G, table 7) was carried out. Out of 12 tetrads recovered from this cross between sibs, seven continued to exhibit a Mendelian, 2 G : 2 g segregation while four showed a Mendelian segregation of two slow and two negative, and one tetrad was 1 G : 1 g_s : 2 g.


SC 1
UNADAPTED
ADAPTED
ADAPTED
X
12475g
X
12475g
X
12475g
LIFE CYCLE IN ABSENCE OF GALACTOSE
18 ASCI g_s g_s g g
1 ASCUS g_s G g g
LIFE CYCLE IN ABSENCE OF GALACTOSE
10 ASCI g_s g_s g g
2 ASCI G G g g
LIFE CYCLE IN 2% GALACTOSE + 2% GLUCOSE
5 ASCI g_s g_s g g
4 ASCI g_s G g g
2 ASCI G G g g
X
LIFE CYCLE IN ABSENCE OF GALACTOSE
7 ASCI g_s g_s g g
6 ASCI G G g g
2 ASCI g_s G g g
X
LIFE CYCLE IN ABSENCE OF GALACTOSE
7 ASCI G G g g
4 ASCI g_s g_s g g
1 ASCUS g_s G g g

DISCUSSION

It is apparent from these experiments that the slow stocks of composite origin developed in this laboratory show a pattern of behavior quite different from the phenomenon of slow fermentation characteristic of the culture of *S. chevalieri* employed in this study. The differences are reflected in the plating experiments and in the results of introducing uniform inocula into a series of fermentation tubes, as well as in the genetical analyses. A striking feature of

SC 1 is the consistency with which it evolves gas on the 5th and 6th day of inoculation in contrast to the long and irregular delays characteristic of 6166. Furthermore, no colonies resembling those 6166 in either size or number appear in pour-plates of *S. chevalieri;* the only resemblance between 6166 and *S. chevalieri* lies in the low orders of variance of the *G* colonies of 6166 on galactose plates and of those of *S. chevalieri* as reported by SPIEGELMAN *et al.* It will be noted, however, that in the present study a roughly 50 percent proportion of the two sizes of colonies of *S. chevalieri* was observed in light seedings (3×10^6) of this species on EMB plates as well as in pour-plates with a cover layer of non-nutrient agar; and, moreover, that the larger colony size could not be correlated with rapid fermentation as reported by SPIEGELMAN *et al.* for isolate $C_1{}^d$ of the same culture.

Even more revealing information is provided by the genetic analyses of both stocks. Notwithstanding the low variance of *G* colonies of 6166 or other clones of similar stocks, a consistent Mendelian segregation of rapid fermenters together with genetical data reported previously confirms the mutant nature of the derived large fermenter colonies of our slow stocks. These data become more significant when we consider that these genetical analyses were made in glucose medium without galactose being involved during matings, isolation of spores or culturing to influence the modification of segregation ratios.

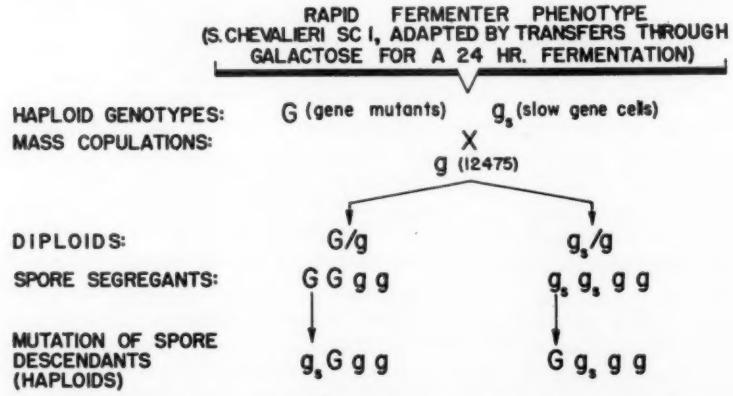
In view of the constancy of the latent period before active fermentation and the genetical analysis involving an unadapted culture (table 4) these experiments prove that slow fermentation by *S. chevalieri* (SC 1) is determined by a specific gene; and that the period of six quiescent days prior to active gas release is due to a physiological state characteristic of the organisms during which the galactozymase concentration is built up to functional levels. These views corroborate the original findings of WINGE and ROBERTS.

It will be observed, however, that stable *G* clones appear in the crosses analyzed in tables 4, 5, 6, and 7. These are similar to the exceptional segregants adapted by WINGE and ROBERTS to rapid fermentation through long continued cultivation in galactose and which failed to revert to slowness when cultivated in galactose-free, wort medium. The crosses reported in the present study were effected by the techinque of mass copulations. Some tetrads resulting from these crosses possess two stable *G* segregants each while others exhibit a 2 : 2 segregation of slows and negatives, suggesting that the original adapted population of SC 1 used in crossing comprised two types of cells: (1) those carrying the specific gene, g_s, determining slow fermentation and (2) initially g_s cells which had become transformed in the direction of 1–2-day rapid fermentation, i.e., of genotype *G*. The mutant nature of the type (2) cells is indicated by their inability to revert and that when intra-ascus crosses between a stable *G* segregant and a negative are made, the resulting asci continue to yield 2 *G* : 2 *g* segregations notwithstanding the fact that the crosses were made in glucose medium (tables 5, 6, and 7). The circumstance that the same intra-ascus cross also yielded 2 g_s : 2 *g* indicates that some of the descendants of the original *G* segregant cells (such as 1196, table 5; 1307, table 6)

back-mutated to the g_s genotype in stock culture on glucose and, in subsequent crosses, copulated with g cells (1194, table 5; 1306, table 6) to give $2\,g_s : 2\,g$ tetrads. Furthermore, we may explain the $1\,G : 1\,g_s : 2\,g$ tetrads encountered in these crosses on the basis that one of the clones initially segregating with the g_s genotype (in a $2\,g_s : 2\,g$ ascus) subsequently mutated to G. The occurrence of one such tetrad (table 6) where an unadapted *S. chevalieri* is involved as a parent, will be noted.

It is evident that the three kinds of tetrads could be detected only by use of the mass copulation technique which permits random matings of cells of different genotypes in a mixed population. That the three types of tetrads could be due to the segregation of a modifier gene appears unlikely in view of the fact that they are encountered only in the crosses involving adapted *S. chevalieri* and in crosses of intra-ascus siblings, while 18 out of 19 asci obtained by crossing unadapted SC 1 are, significantly, of the type expected on the basis of a single gene (g_s, g) difference.

Judged on these bases, one may interpret rapid fermenter phenotypic segregants that are incapable of deadaptation to be constituted of mutant (G) cells some of which can revert to the g_s type and which, during mass matings, fuse with g cells (12475) just as do the genotypically G cells. This explanation as to the origin of $2\,G : 2\,g$; $1\,G : 1\,g_s : 2\,g$; and $2\,g_s : 2\,g$ tetrads recovered from an intra-ascus cross between a stable G culture and g culture is diagrammatically represented below.



From their recent experiments with long-term adapted *S. chevalieri*, involving a study of the reversion of the positive to the negative phenotype, by means of serial isolation and characterization of buds produced by positives in the absence of substrate, SPIEGELMAN, DE LORENZO and CAMPBELL (1951) demonstrated the existence of cytoplasmic elements. These experiments involved a very few cells and a restricted number of cell divisions. It may be observed here that in the present study involving large populations and a high

order of cell generations, a similar cytoplasmic explanation may be invoked only in those cases where reversions can be achieved. The present experiments establish, moreover, that when the same organisms are actively multiplying and yield large populations, mutations may be easily detected through genetical analysis and provide an explanation for the existence of occasional clones which are incapable of reversion.

SUMMARY

Different modes of long-term adaptation to the rapid fermentation of galactose are exhibited by two different types of Saccharomyces haploids. Cultures of the first type, of composite genetic origin, ferment galactose after long and irregular delays, achieving adaptation by the production of rapid fermenter variant cells in initially non-fermenter populations. These variants are detected as large colonies in galactose pour-plates and occur in low frequencies and with a low order of variance. The mutant nature of these rapid-fermenter variants is proved by their inability to revert in the absence of substrate and by the fact that on outcrossing them with non-fermenter cells, asci yielding consistently Mendelian segregations of two rapid-fermenter and two non-fermenter progeny are obtained.

The second type, a culture of *Saccharomyces chevalieri*, achieves long-term adaptation consistently on the sixth or seventh day of contact with galactose and is determined by a slow gene. However, long-term adapted clones revert if contact with galactose is broken. Occasional stable, adapted, rapid-fermenter clones are encountered, however, which fail to revert in the absence of substrate. Genetic analyses have shown that such exceptional clones originate through mutation and selection in the presence of substrate.

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A CYTOGENETIC STUDY OF THE Y CHROMOSOME IN DROSOPHILA BUSCKI *

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IN contrast to other species of Drosophila studied in this respect, primary non-disjunction of the sex chromosomes of *Drosophila buscki* females results in the appearance of XXY females but no XO males. In various matings designed to detect primary non-disjunction, 73 exceptional females and no exceptional males were found among 85,469 flies examined (KRIVSHENKO 1941a). Special experiments have shown that gametes devoid of the sex chromosomes are viable and functional. The absence of XO males indicates, then, that the Y chromosome of *D. buscki* contains a gene or genes which are essential for the development of zygotes which carry a single Y chromosome (KRIVSHENKO 1941b, c). Examination of the chromosomes in the salivary gland cells of male larvae of *D. buscki* has revealed the presence of an euchromatic element consisting of some 14–15 discs, which has been identified as the euchromatic part of the Y chromosome (SIROTINA 1938; KRIVSHENKO 1939). Further studies of the Y chromosome in *D. buscki* are now reported.

MATERIAL AND METHODS

A strain of *D. buscki* derived from flies collected at Columbia, Missouri, by PROF. A. B. GRIFFEN, has served as initial material for our study. Other strains examined, especially for metaphase chromosome configurations, came from Verdi, Nevada (obtained through the courtesy of the University of Texas), from Liberton, near Edinburgh, Scotland (sent by DR. J. M. RENDEL), from Belluno, Italy (furnished by PROF. A. BUZZATI-TRAVERSO), from Tokyo, Japan (obtained from PROF. D. MORIWAKI), and from São Paulo, Brazil (sent by DR. H. STALKER, Washington University, St. Louis, Missouri).

Mutations were obtained chiefly in the offspring of males from the Columbia strain treated with 4200–4500 r units of X-ray. The mutants were examined for possible associated chromosomal aberrations; whenever aberrations were found, the breakage points were located with the aid of the "map" of the salivary gland chromosomes published by SIROTINA (1938). In all, 12 visible mutants and 5 chromosome aberrations associated with the Y chromosome were found. Of these, the five visibles described below were used for the present work.

The flies were raised on a medium prepared from the following ingredients: water 1000 cc, agar 10 g, corn meal 63 g, brewer's yeast dried 63 g, corn molasses (Karo) 100 g, moldex (10% sol.) 5 cc. The cultures were kept at room temperature (22–26°C).

NORMAL CHROMOSOMAL COMPLEMENT OF D. BUSCKI MALES

After the early work of METZ (1916), the first detailed study of the chromosomes of *D. buscki* was published by M. SIROTINA (1938), who used flies collected by the present writer at Kiev, Ukraine. According to SIROTINA, the chromosome complement in the nerve cells of the larval brain of males of this species consists of two pairs of chromosomes with median centromeres, an acrocentric X chromosome, and a metacentric Y chromosome. The X chromosome sometimes shows a spherical " satellite " at its centric end, thus revealing that its centromere is subterminal, and that it consists of a long " left " arm and a short " right " arm. The Y chromosome is V-shaped, the two arms showing a length ratio of about 1 : 1.09. The combined length of the two arms of the Y chromosome is about 2.3 μ, while that of the X chromosome is about 1.5 μ. The ratio of the entire length of the Y chromosome to the X chromosome is thus about 1.5 : 1. The salivary gland chromosomes were found by SIROTINA to consist of seven elements, five of them long and two short. Among the five long elements, four are darkly staining, and correspond to the two pairs of autosomes. The fifth long strand is pale, and corresponds to the X chromosome. The short elements, which in many nuclei appear to be together, represent the Y chromosome and the " right " arm of the X chromosome. The length of the Y is about 6 μ, which amounts to 4–5 percent of the length of the X chromosome. The XR is still shorter, about 4 μ, and contains approximately 11 discs.

In general, SIROTINA's description of the salivary gland chromosomes of the Kiev population fits the conditions found in the American representatives of the species. But the chromosomes in the ganglion metaphases show significant differences in the shapes and lengths of the sex chromosomes. The Y chromosome of the males in populations examined by the writer is rod-shaped, and often shows a small knob-like thickening at the centromere end. Between this knob-like thickening and the long distal part of the Y chromosome a slight constriction can sometimes be observed. The X chromosome in the males of these populations is rod-shaped, or more correctly, saber-shaped. A barely discernible constriction can be observed at about one-quarter to one-third of the length from the proximal end. This is, however, not a centric constriction, because the chromosome always lies with its proximal end directed towards the center of the equatorial plate. The condition of the right arm of the X chromosome observed by SIROTINA has never been encountered in the material examined by the present writer. The ratio of the length of the Y and the X chromosomes in the males is 0.85 : 1. Thus, the Y chromosome of *D. buscki* males observed by the writer is about 75–80 percent shorter than the Y chromosome of males from the Kiev population (figure 1).

The relatively short Y chromosome has been found in all the stocks used for the present study—those from Missouri, Nevada, Scotland, Italy, Japan and Brazil. Since there is no reason to doubt the accuracy of the data of SIROTINA (1938), it appears that two distinct types of Y chromosome exist

in *D. buscki*. A similar situation has also been found in *D. pseudoobscura* and *D. persimilis* by DOBZHANSKY (1935, 1937).

DESCRIPTION OF THE MUTANTS AND THEIR CHROMOSOMES

1. Spotless-Y (186); *(Sp-Y; 30/VI 1949)*. The males have a slight reduction of the lateral spots on the abdomen, whereas all females are normal in this respect. The fertility and the viability of the mutants are comparatively high, the male–female ratio being approximately 1 : 1.2. Among the males, there sometimes appear fertile individuals of a second class which have a somewhat shortened body. The pigmentation of their bodies is somewhat paler, and

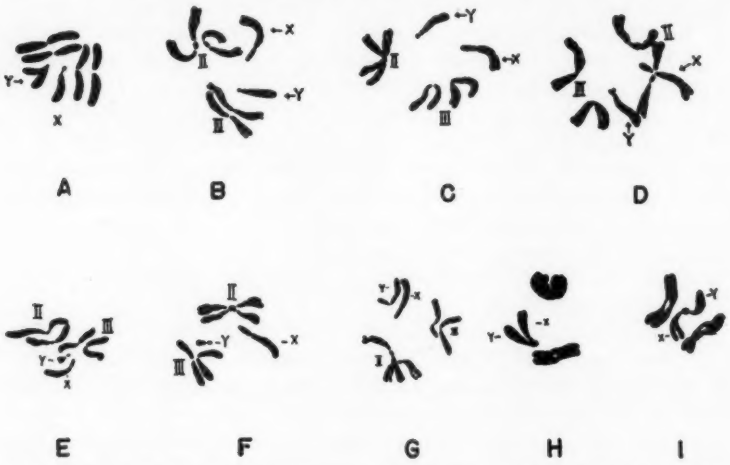

FIGURE 1.—A-D, Metaphase chromosomes of the males of *D. buscki* from different populations: A, Kiev, Ukraine (according to SIROTINA 1938; B, Columbia, Missouri; C, Tokyo, Japan; D, São Paulo, Brazil; E and F, Metaphase chromosomes of (E) euploid and (F) aneuploid males in mutant Curled-Y; G, Metaphase chromosomes of the males in mutant Echinus-Y; H and I, Somatic pairing of chromosomes in ganglion cells of the males.

their eclosion is delayed for two days. In their progeny there appear 15–20 percent males of the paternal type.

A cytological analysis of the salivary gland nuclei of the mutant males (first class) revealed a break in the distal part of IIR, approximately in the fifth section. The distal piece of the aberrant chromosome usually pairs completely with its normal partner; but sometimes it does not pair in its lowest part. The proximal piece always pairs completely. In the males of the second class, the distal piece of the IIR is a trivalent. Sometimes the third short element, the lower end of which coincides with the region of the break in IIR in the males of the first class, does not pair at all. Then, it can be found somewhere closer to the center of the nucleus as a small univalent, usually near the proximal

ends of the other chromosomes. The euchromatic Y chromosomal element in salivary gland cells of males in both classes does not change and does not take any part in the aberration. The chromosomes of the ganglion metaphase do not reveal any visible change in males of either class.

The patroclinous character of the inheritance of this mutant indicates that it is associated with the Y chromosome. The presence of the definite chromosomal aberrations in the males of both classes also indicates that these aberrations are connected with the Y chromosome, though we did not succeed in determining the nature of this connection by direct observation. A simple explanation of this situation is that a reciprocal translocation between IIR and the Y chromosome has occurred. The observed normality of the metaphase chromosomes is, then, due to the fact that during the process of reciprocal translocation approximately equal parts of the chromosomes have been exchanged. The absence of a visible connection between the distal part of IIR and the Y chromosome in salivary gland nuclei indicates that the break in the Y chromosome took place somewhere in the heterochromatic region. Since the proximal end of the Y chromosome, which is well marked by a knob-like thickening, remains unchanged, the break in this chromosome must have taken place somewhere in its distal part. On the basis of the fact that the metaphase chromosomes remained unchanged, and considering that five sections of IIR equal approximately to one-fifth of the length of this II-chromosome arm, it may be inferred that the break in the Y chromosome occurred at about the same distance from the distal end (figure 2 B). The appearance of the males of the second class is easily understood; these males are hypoploid for the distal part of the Y chromosome and hyperploid for the distal part of IIR (figure 2 C).

2. Delta-Y (*356*); (*Dl-Y; 15/VIII 1949*). The males have widened distal ends of the radial and medial veins of the wings. Fertility and viability of the mutant are high, for the male–female ratio is 1 : 1.15. Among the progeny of Delta-Y males there sometimes appear males without the Delta phenotypic effect. They usually appear on the second or the third day from the beginning of hatching. These males have somewhat shorter body and frequently hold their wings out to the side as in the mutant Dichaete. The wings may also be inflated with liquid. The males of this second class are also fertile and in their progeny appear about 15 percent males that resemble their fathers; their viability is significantly lowered.

A cytological analysis of the salivary gland nuclei has revealed a complicated chromosomal aberration involving both IIR and IIL. In IIR two breaks occurred, the first at the boundary of the seventh and eighth sections and the second break in about the twelfth section. As the result of these breaks, three pieces were formed: a distal, a middle, and a proximal one. The middle piece attached itself in an inverted position to the top of the proximal piece of IIR; the distal piece did not join the middle piece. As a result of this, IIR formed a loop in its middle part. The distal piece usually does not conjugate in its lower part with its normal partner; occasionally it is free and located somewhere near the proximal ends of the other chromosomes. Four breaks occurred

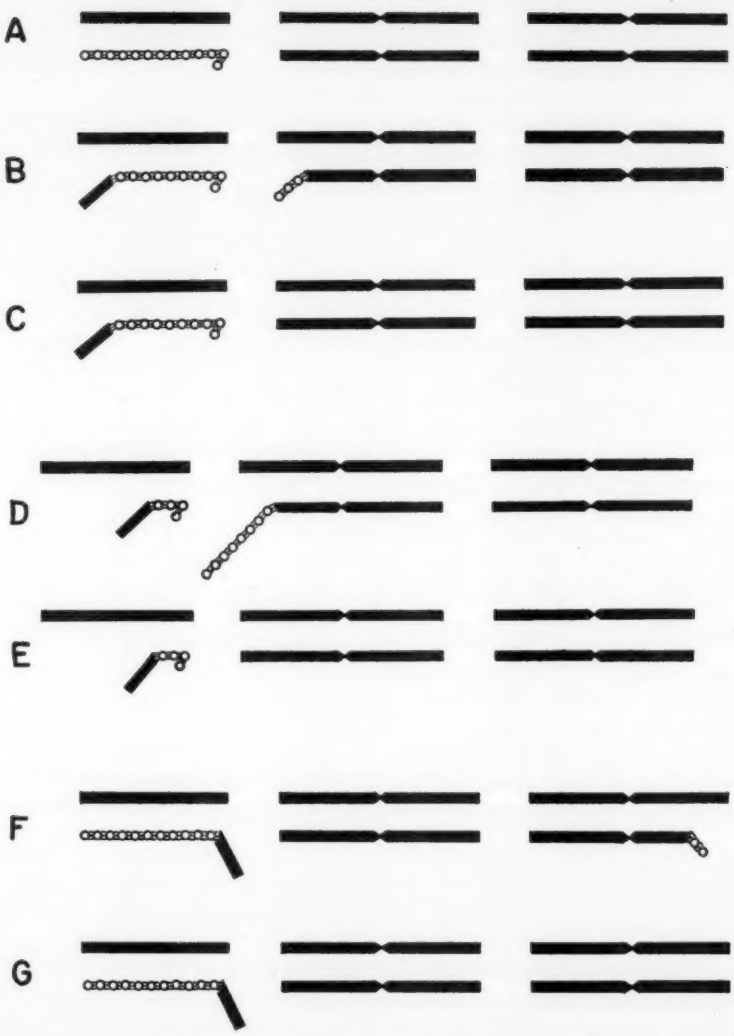

FIGURE 2.—Schematic structure of the chromosome complements: A normal males of wild stock; (B) euploid and (C) aneuploid males in mutants Spotless-Y (186) and Delta-Y (356); (D) euploid and (E) aneuploid males in mutant Curled-Y (404); (F) euploid and (G) aneuploid males in mutant Echinus-Y (507/1).

in IIL and five pieces were formed. These later joined together again in a different order making a complicated figure similar to that of an overlapping inversion. The first break occurred in the third section; the second, the third and the fourth breaks took place approximately in the thirteenth, eighteenth and twenty-first sections, respectively. In the males of the second class a trivalent of the distal end of IIR is always observed. The lower end of the third element borders the place of the distal break of IIR in the males of the first class. Sometimes this element does not pair; then it is located near the proximal ends of the chromosomes and does not reveal any connection with the euchromatic element of the Y chromosome. The chromosomal aberrations found in males of the first class is absent in this case. The euchromatic element of the Y chromosome of the salivary gland nuclei remains without any visible change in both classes of males, and does not take any part in the chromosomal aberration. The metaphase chromosomes in the ganglion cells of males of the first class seldom give good configurations, but when they do, it is impossible to discover any change in the relative lengths of the chromosomes concerned. The metaphase chromosomes in males of the second class are also normal in appearance.

The inheritance of the characteristic Delta through the male line, from father to son, indicates its connection with the Y chromosome. The absence of a normal Y chromosome coupled with constant presence of a complicated chromosomal aberration in males of the first class, and of the third element of the distal part of IIR in males of the second class, also indicate the connection of this phenomenon with the Y chromosome. We did not succeed in establishing the involvement of the Y chromosome by direct observation, because the visible euchromatic element of the Y chromosome in salivary gland cells is not included in the aberration, and because the metaphase chromosomes do not differ from normal in their appearance. However, the cytogenetical nature of this mutant line can be explained if it is assumed that a reciprocal translocation between the second autosome and the Y chromosome has taken place. The fact that the distal piece of IIR in the males of the first class is not connected with the rest of IIR, and that the same element is constantly present in males of the second class in addition to the normal autosomal complex, indicates the connection of this distal piece of IIR with the Y chromosome. The lack of a visible connection of IIR with the euchromatic part of the Y chromosome indicates that attachment has occurred in the heterochromatic region of the Y chromosome.

Since the distal piece of IIR broke between the seventh and eighth sections, the size of this piece is approximately one-fourth the length of IIR. If this segment were translocated to the proximal end of the Y chromosome, it should be visible in the metaphase chromosomes. But in not a single instance has a change been discovered in the structure of the proximal end of the Y chromosome. The distal part of IIR appears to be translocated to the distal end of the Y chromosome, with a reciprocal translocation of a piece from this region

of the Y chromosome to the free end of the middle, inverted piece of IIR. The absence of a visible change in the length of the metaphase Y chromosome, or in the autosomes, indicates that approximately equal pieces have been exchanged (figure 2 B and C).

3. Curled-Y (404); (Cu-Y; 28/X 1949). A majority of the males have the ends of their wings bent upwards. Other males have their wings crumpled or normal. The degree of penetrance and expressivity of the Curled trait in a great degree depends upon external and genotypical conditions. The fertility and the viability of the mutants are high, the male–female ratio being 1 : 1.1. Among the offspring of the mutant males about 10–15 percent of the males appear with a somewhat shortened body and with wings which are sometimes distended with liquid. The males of this second class are completely sterile, and their viability is low. They usually appear on the second or the third day of general hatching. In the progeny of the mutant males there very seldom appear females of the Curled type, and still less frequently females are found which appear similar to the second class of the males. The females of the first type possess a high fertility and viability, while those of the second type are much less fertile and viable.

Cytological analysis of the salivary gland chromosomes revealed that IIR has been broken at the end of the seventh section. The proximal part of the broken IIR usually pairs fully with its unbroken partner, while the newly formed distal piece of this broken chromosome does not pair in its lower part with the normal univalent. Sometimes it does not pair at all, being located somewhere near the proximal ends of the other chromosomes or near the nucleolus. Fairly often larvae are found in which the distal part of IIR is of a trivalent structure. In this case, the third element is equal to the distal piece of the aberrant IIR present in mutant males. Apparently larvae of this type give rise to adult males of the second class. The euchromatic part of the Y chromosome in salivary gland cells remains without any visible change in both classes of males, and does not show any connection with the aberration. In ganglion metaphases of males of the first class, the Y chromosome is represented by a short two-armed chromosome approximately one-third as long as a normal one. One arm of the new Y chromosome is somewhat longer than the other. The shorter arm apparently represents the proximal, knob-like thickening of the normal Y chromosome. One of the autosomes also has a noticeably longer arm (figure 1 E). In anaphases, even at late anaphase, these chromosomes often form bridge-like figures. In ganglion metaphases of male larvae whose salivary gland chromosomes possess a trivalent, all the autosomes have a normal appearance. The Y chromosome in this case is also short and two-armed (figure 1 F). In the ganglion metaphases of the mutant females the same changes are observed as in the corresponding classes of males.

The cytological and genetic data indicate that a reciprocal translocation between IIR and the Y chromosome has taken place. A break in IIR has occurred in the seventh section, and the distance from the break to the distal end of this chromosomal element is approximately equal to one-fourth of its

length. Apparently this break is also located in the metaphase chromosome at approximately the same distance from its distal end. This interpretation is supported by numerous observations on various chromosomal aberrations, which permit the conclusion to be drawn that the heterochromatic regions of the proximal ends of the autosomes in *D. buscki* are so small that they do not produce marked differences in the comparative length of the chromosomes in ganglion or in salivary gland cells. The break in the Y chromosome occurred in its heterochromatic proximal part, not far from its knob-like end. It should be noted that the length of the newly formed Y chromosome in this mutant is about 1/3 normal, and that the length of the distal piece of IIR, which has become a part of this Y chromosome, is about 1/4 of IIR (the length of which is approximately equal to the length of the normal Y chromosome in ganglion cells). It follows that the distance from the break to the proximal end is about 1/12 of the length of the Y chromosome. Since the euchromatic element is not involved in this aberration, it can be inferred that it, together with the centromere, has remained in the unchanged proximal piece of the Y chromosome. The correctness of this analysis has been confirmed by direct observation of a reciprocal connection between IIR and the heterochromatic part of the Y chromosome (see below).

Thus the mutant males of the type Curled-Y (first class) have a reciprocal translocation between Y and IIR (euploid males); the males of the second class are hypoploid in the long distal part of the Y chromosome and hyperploid in the distal part of IIR. The appearance of the mutant females as well as of females whose type is similar to that of the aneuploid males can be explained by non-disjunction of the sex chromosomes in the males (figure 2 D and E).

4. Minute-Y (236); (M-Y; October 1937). The males have thin, slightly shortened macrochetae. The fertility and the viability are lowered. Among the offspring of the mutant males, about 11 percent of the sons have normal bristles, but darker and slightly roughened eyes. The males of this second class are completely sterile (KRIVSHENKO 1939).

A cytological analysis of the salivary gland cells in males of the first class has revealed a reciprocal translocation between the euchromatic element of the Y chromosome and IIIL. The euchromatic element of the Y chromosome broke close to the proximal end, and IIIL broke in section 8 or 9. The distal part of the euchromatic element of Y became translocated to the proximal part of IIIL, and the distal part of IIIL became reciprocally translocated to the proximal part of the euchromatic element of the Y chromosome.

Unfortunately there are no data concerning the chromosomal structure of the ganglion metaphases or of the salivary gland cells for the males of the second class; these males are presumably hypoploids for a part of the Y chromosome, and hyperploids for the distal part of IIIL.

5. Echinus (507/1); (Ec-Y; 9/I 1950). The males have eyes with slightly enlarged hairs, and irregularities in the distribution of the facets are sometimes observed. The fertility and viability of the males are high, for the male–female

ratio is 1 : 1.3. Only one class of mutant males appears and very seldom females of the mutant type also are found.

Analysis of the salivary gland cells revealed a translocation of the euchromatic part of the Y chromosome to IIIR, IIIR having broken in approximately the tenth section. The whole visible euchromatic part of the Y has become translocated to the proximal part of IIIR (figure 4, upper right). The break in the Y chromosome apparently occurred in the region of the last proximal discs. The distal part of IIIR sometimes pairs completely with its normal partner; in other cells it pairs only at the top, while its lower end lies in the vicinity of the proximal ends of the other chromosomes, usually close to XR. Very often XR and the distal part of IIIR are joined close to their proximal ends. Several times the distal part of IIIR was observed to be completely free. In ganglion metaphases the aberrant Y chromosome is two-armed, and its longer arm is long and is in fact the unaltered part of the Y chromosome. The other arm is short, its length being equal to about one-third (or somewhat longer) of the first arm (figure 1 G). One of the autosomes has an arm approximately as much shortened as the second arm of the Y chromosome has been lengthened. Frequently metaphase plates occur in which the short arm of the Y chromosome is located close to the normal arm of the third chromosome, the partner of which has a shortened arm. In these cases a definite attraction of the homologous elements is observed.

A reciprocal translocation is involved in this mutant. As result of this translocation, the knob-like ending of the proximal end of the normal metaphase Y chromosome has been replaced with a large element, which undoubtedly represents the distal part of IIIR. The euchromatic element which has been translocated to the top of the proximal part of IIIR represents the knob-like thickening of the normal Y chromosome. In ovarian cells of the rare mutant females, the same aberrant Y chromosome and autosome have been detected. The occurrence of these females is due to primary non-disjunction of the sex chromosomes in the mutant males (figure 2 F and G).

DISCUSSION AND CONCLUSIONS

As mentioned above the normal Y in the ganglion metaphase of males of *D. buscki* is a rod-shaped chromosome having a small knob-like thickening at the centromere end. The length of this chromosome is equal to about 85 percent of the X chromosome. In salivary gland cells the Y chromosome is represented by a small euchromatic element equal to about 4–5 percent of the length of the polytene X chromosome. Consideration of these data, showing the comparative length of the Y chromosome in the different cells of the males, would seem to lead to the conclusion that the metaphase Y chromosome consists mainly of heterochromatin invisible in salivary gland cells.

The above data, however, do not enable a decision to be made whether the small euchromatic element of salivary gland cells corresponds to a proportionally insignificant part of the mitotic Y chromosome, nor how euchromatic and heterochromatic parts are distributed in the mitotic Y chromosome, nor,

lastly, whether the genetic activity of the Y chromosome resides only in the region of the euchromatic element. It is nevertheless possible to find a comprehensive solution to all these questions by careful cytogenetical analysis of the aberrations associated with the Y chromosome which have been described above.

1. *The location of the heterochromatic and euchromatic portions in the metaphase Y chromosome*

The mutants associated with the Y chromosomes can be divided into two groups. To the first group belong Spotless-Y, Curled-Y, and Delta-Y; to the second group belong Minute-Y and Echinus-Y. In Spotless-Y and Delta-Y, the Y chromosome has been broken in the distal portion of the heterochromatic part. In Curled-Y, the break also took place in the heterochromatic part, but in the proximal portion thereof, and not far from the knob-like end. The knob-like end in all these lines remains unchanged. On the other hand, the chromosomal fractures in Minute-Y and Echinus-Y took place close to the proximal end of the euchromatic part of the Y chromosome, as can be seen in the salivary gland chromosomes.

In the mutants of the first group, no break took place in the euchromatic portion of the Y chromosome. The euchromatic portion, together with the translocated distal piece of IIR, has formed the new Y chromosomes which are responsible for the mutant phenotypes of the euploid and aneuploid males of these lines. No direct connection is observed between the distal parts of the IIR and the euchromatic portion of the Y in the salivary gland chromosomes. This shows that a section of the heterochromatic portion of the Y chromosome, differing in length in the different mutants, is intercalated between the translocated part of IIR and the euchromatic part of the Y chromosome. The intercalated portion is relatively long in Spotless-Y and Delta-Y, and relatively very short in Curled-Y.

In Minute-Y and Echinus-Y, the translocated parts of the Y chromosome include all or nearly all of the euchromatin. These parts always show a connection with one of the arms of the third chromosome, and no connection with the remaining heterochromatic part of the Y. This heterochromatic part retains its centromere, however, and it now plays the role of the partner of the X chromosome in meiotic segregation. In metaphase plate preparations of Echinus-Y, the Y chromosome does not show a knob-like thickening of the centromere end. The latter is replaced by another arm that is doubtless the translocated distal portion of the IIIR. The long distal part of the Y chromosome has suffered no change in this mutant. The changes in Minute-Y are presumed to be analogous with those of Echinus-Y.

The structure of the newly-formed Y chromosomes in the lines Spotless-Y, Delta-Y, and Curled-Y, on the one hand, and in the lines Minute-Y and Echinus-Y on the other hand, enable us to conclude that the Y chromosome of *D. buscki* is a two-armed chromosome with its centromere located between the euchromatic and heterochromatic parts. The long rod up to the knob-like

thickening represents the " left " arm, consisting of heterochromatin. The small proximal knob-like ending represents the euchromatic " right " arm. The small euchromatic element seen in salivary gland cells is the knob-like thickening itself, that is, the right arm of the metaphase Y chromosome. The definite location of the centromere is for the time being unknown, but on the basis of cytological data regarding the mutant lines Curled-Y and Echinus-Y it is possible to conclude that it is located close to the knob-like ending and, very possibly, on the border of the heterochromatic and euchromatic parts of the Y chromosome (figure 3).

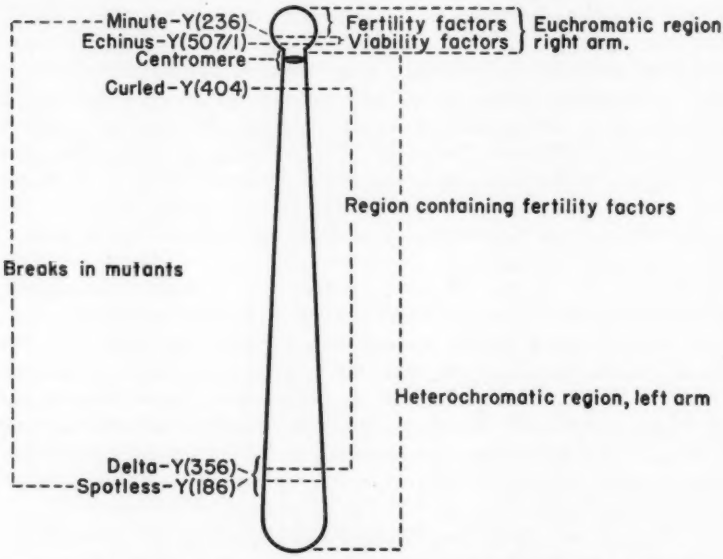

FIGURE 3.—Structure of the Y chromosome in somatic metaphase of *D. buscki*.

2. *The roles of the various sections of the Y chromosome in viability and fertility of the male*

The five mutants discussed above all have been shown to be connected with translocations involving the Y chromosome and one of the autosomes. Theoretically the phenotypic characteristics of these mutants could be caused either by loci which normally lie in the Y, or in the autosomes, or in both. It seems most likely that the loci responsible for these mutant characters are originally autosomal. This is made especially probable by the fact that the phenotypic characteristics of the mutants fail to appear in the aneuploid males in which the aberrant recipient autosomes are absent.

The three mutants of the first group, Spotless-Y, Delta-Y, and Curled-Y, are very different in phenotype. Nevertheless, the aneuploid males which arise

in the progeny of these mutants are fairly similar phenotypically. All of them have shortened bodies, lowered developmental rates, and decreased viability. These characteristics of the aneuploid males are absent in the euploid mutants having reciprocal translocations. It follows, then, that the phenotypes of the aneuploid males are modified either by the deficiencies of the heterochromatic portions of the Y chromosome or by the duplications for the distal end of IIR.

To discriminate between these possibilities, males were obtained from the lines Curled-Y and Delta-Y which carried the translocated Y chromosome in addition to a normal Y chromosome (XYY'). Despite the presence in these males of a normal Y, they resemble the aneuploid males in phenotype. This phenotype is, then, due to autosomal duplications. It may be noted that the duplicated portion of the IIR is shortest in Spotless-Y, is longer in Curled-Y, and is longest in Delta-Y. The degree of departure from the normal phenotype is, correspondingly, smallest in Spotless-Y, greatest in Delta-Y, and intermediate in Curled-Y. The aneuploid males obtained in the progeny of Minute-Y are quite different in phenotype from the aneuploids of the first group of mutants. This is, indeed, not unexpected, since the aneuploids obtained from Minute-Y contain a duplication for a section of the IIIL chromosome, instead of IIR.

Although the phenotypic characteristics of the above mutants are not produced by changes in the loci carried in the Y chromosome, there can be no doubt that the Y of *D. buscki* is very important for the viability and fertility of the flies. It is relevant in this respect that the aneuploid males obtained from Spotless-Y and Delta-Y are fertile, while those descended from Curled-Y are sterile. The aneuploids coming from Curled-Y carry a deficiency for a much longer section of the left arm of the Y chromosome than the Spotless-Y and Delta-Y aneuploids. On the other hand, the Curled-Y and Delta-Y aneuploids contain duplications for approximately the same sections of the IIR chromosome. It follows that the loci concerned with male fertility lie in the heterochromatic portion of the Y chromosome, between the breakage points observed in Curled-Y and Delta-Y (figure 3). More precise localization of these loci is impossible on the basis of the information now available. According to NEUHAUS (1939) the fertility factors in the Y chromosome of *D. melanogaster* are localized in the distal portions of both limbs of that chromosome. In *D. buscki*, on the other hand, these factors seem to be contained in the middle part or, more likely, towards the proximal part of the left arm of the Y chromosome.

Aneuploid males derived from Minute-Y, in which about three-fourths of the distal portion of the right arm of the Y chromosome is absent, are absolutely sterile. Finally, the aneuploid males do not appear at all in the progeny of Echinus-Y. In such males almost the entire euchromatic section of the Y would be absent. To be sure, such aneuploids would also carry a fairly long duplication for a section of IIIR. But it is probable that their lethality is due primarily to the deficiency for the centromeric region of the right arm of the Y chromosome. Thus a deficiency for the distal portion of the heterochromatic

limb of the Y permits viability as well as fertility to be retained. A longer deficiency (Curled-Y) produces viable but sterile males. A male having deficiency for the distal portion of the right (euchromatic) arm (Minute-Y) is also viable, but it is sterile. Finally, a deficiency for most of the right arm (Echinus-Y) is lethal (figure 3).

The viability of males deficient for most of the left arm of the Y chromosome suggests that this arm is genetically fairly inert. No data are available for the portion of the left arm lying between the break in Curled-Y and the centromere (figure 3). No mutant genes are known in the euchromatic right limb of the Y, and males deficient for the section between the break in Minute-Y and the free end of this chromosome are viable but sterile. And yet a deficiency for a longer section, between the break in Echinus-Y and the free end, is lethal (figure 3). This suggests that the portion of the right arm adjacent to the centromere is in fact genetically active.

In *D. melanogaster* XO males are viable but sterile. Males with a deficiency for either arm of the Y are also sterile (STERN 1929). It may be that the left arm of the Y chromosome of *D. buscki* is in part homologous to the Y of *D. melanogaster,* but the right arm of the Y of *D. buscki* is a genetically active element absent from the Y of *D. melanogaster* and also from other Drosophila species so far investigated in this respect.

3. *The origin of the right arm of the Y chromosome in D. buscki*

D. buscki is remarkable among its congeners by its possession of a Y chromosome which contains an euchromatic part visible in the salivary gland cells and which is genetically active. The origin of this condition may be interpreted in two ways. (1) The euchromatic right arm of the Y chromosome may be a relic of an ancestral condition in which the entire chromosome was euchromatic and genetically active. (2) The euchromatic arm may have arisen by translocation of genetically active material to the Y chromosome from some other chromosome of the ancestral complement. Since retention of an ancestral euchromatic condition of the Y chromosome in only a single species of the genus seems improbable, it appears at the present time that the second interpretation is the more reasonable one.

D. buscki is one of the relatively few known species of the genus which lacks the microchromosomes in the chromosome complement (corresponding to the fourth chromosomes of *D. melanogaster*). KRIVSHENKO (1939) and WHARTON (1943) accordingly suggested that the right arm of the X chromosome is homologous to the microchromosomes of most other species, having presumably suffered translocation to the X chromosome in the course of phylogeny.

In *D. buscki* the Y chromosome as well as the X chromosome has a short euchromatic right arm. To be sure the right arm of Y is more elongated, and appears to contain a greater number of stainable discs than a similar element in the X chromosome. Nevertheless, in the salivary gland cells these two elements have often been seen connected by their proximal ends. Sometimes this

association is so intimate that it is no longer possible to find a dividing line between these elements (figure 4, upper left). Lateral pairing between the two elements has been seen only once, however, and it is not certain whether in this one case a true pairing or an accidental juxtaposition was involved.

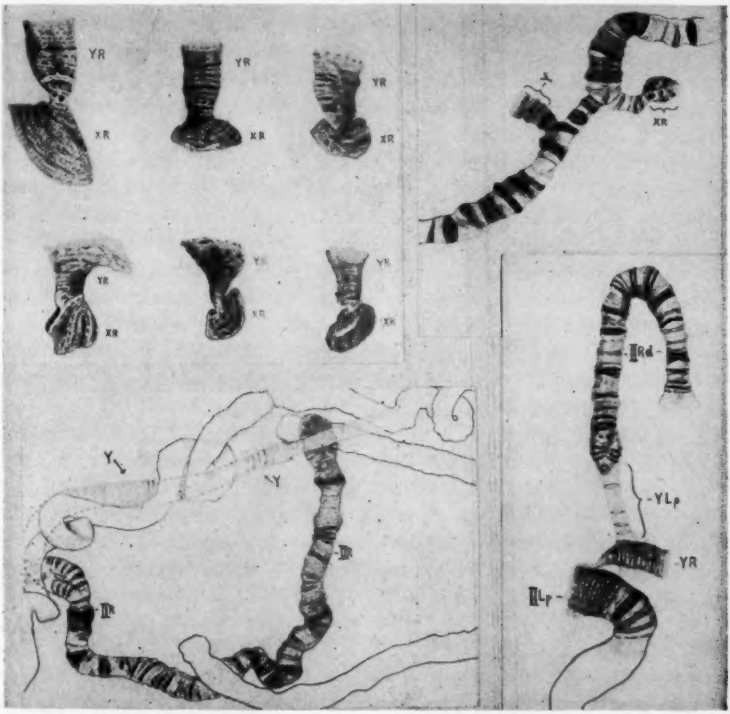

FIGURE 4.—UPPER LEFT, different cases of association between YR and XR in salivary gland cells of the males of *D. buscki*. UPPER RIGHT, a translocation of the euchromatic element of the Y-chromosome to the IIIR in mutant Echinus-Y (XR is connected to the lower end of the distal portion of the IIIR which is translocated to the proximal part of the euchromatic element of the Y). LOWER LEFT, translocation involving the left arm of the Y and the right arm (IIR) of the second chromosome in mutant Curled-Y (this figure represents a tracing of photograph). LOWER RIGHT, translocation involving the distal portion of the IIR and the proximal portion of the left (heterochromatic) arm of the Y chromosome in mutant Curled-Y (IIRd, distal portion of the IIR; YLp, proximal portion of YL; YR, right arm of the Y. The connection between YLp and YR is accidental).

Somatic pairing of chromosomes is pronounced in the ganglion cells of *D. buscki*. In the ganglion cells of the male, somatic pairing between the X and Y chromosomes is usually absent; nevertheless, in some cells an association of the centromere-carrying parts of these chromosomes has repeatedly been

seen. In these instances the X and Y chromosomes often form an angle, the apex of which is occupied by the associated proximal parts of the two chromosomes (figure 1 H). In other cells the two chromosomes are arranged in a straight line, the associated centromeric regions of both forming a thickening in the middle (figure 1 I).

The attraction exhibited by the XR and YR in the salivary gland cells, as well as in ganglion cells, is indicative of at least a partial homology of their structure. This is also confirmed by the behavior of the distal part of IIIR when translocated to YR. For example, it has very often been observed in the Echinus-Y translocation that the right arm of the X chromosome (XR) is associated with the distal part of the IIIR which is continuous with the proximal part of the YR (figure 4, upper right). Taken together these facts seem to indicate that the right arms of the X and Y chromosomes contain some homologous loci, presumably as a result of their common origin. Their complete identity, however, was lost in the process of evolution, and at present indications of their initial likeness is retained chiefly in their proximal parts.

Accordingly the view that XR, as well as YR, is derived from the microchromosomes which are free in other species of Drosophila is at present the most probable one. The simplest interpretations would seem to be that translocations of microchromosomes to the X and Y chromosomes took place stepwise and very soon after one another or, by translocation of the microchromosome to either sex chromosome (X or Y) first, and then transfer to the other as a result of X–Y crossing over. Thus, loci, which in other species are located in the microchromosomes, are present in equal dose in both sexes of *D. buscki*. This hypothesis has been developed on the basis of cytological findings. Recently genetic evidence in its favor has come to light; this evidence will be presented in another publication.

4. *The structure of the heterochromatic part of the Y chromosome of D. buscki*

Although a translocation involving the Y and the IIR chromosomes has been established cytogenetically in the mutant Curled-Y, a physical connection between these chromosomes at first appeared to be absent in the salivary gland chromosome preparations of this mutant. After much effort a chromosomal element, quite different in appearance from the other chromosomes in the same cell, was observed attached to the proximal portion of the IIR involved in the translocation. The most striking peculiarity of this element is its extraordinary paleness. There is no doubt that this lightly staining body is a portion of the Y chromosome, because only a section of the Y chromosome could be attached to the tip of the proximal part of IIR in the Curled-Y mutant. Although this element, because of its poor stainability, can be seen in only a small minority of cells, enough data were eventually obtained (KRIVSHENKO 1950).

In figure 4, lower left (see also KRIVSHENKO 1950), the distal translocated part of the Y chromosome in its most complete form is shown. The length of this element, as seen in the figures, is about 65–75 percent of the length of the

X chromosome in the salivary gland nuclei. Its width in the middle part is about equal to that of other paired chromosomes. Toward the ends, the width is smaller and approaches that of a univalent, euchromatic chromosome. Its distal end has no resemblance to any of the other chromosomes in the salivary gland cells of *D. buscki*. The bands are pale, barely visible, often dotted, and all of them single. The spacing of the bands along the length of the chromosome is about the same as that of the bands of the euchromatic parts of other chromosomes. Because of the generally uniform staining of the individual bands, they appear to be also more uniformly distributed along the length. The contour line of this element, except for two symmetrical arm-like projections at its distal part, is completely smooth. This element is very easily deformed and disintegrated by the least pressure of the coverslip. It should be noted here that this part of the Y chromosome has been observed with normal optics as well as with a phase-contrast microscope. Through the phase microscope the same peculiarities of the structure of this element were observed as in stained preparations.

The proximal portion of the left arm of the Y chromosome, on which the distal part of the IIR was translocated, appears cytologically like its distal portion. In figure 4, lower right, the proximal portion can be seen attached to the base of the translocated section of the IIR chromosome. In this case the part of the Y chromosome appears to be rather short and narrow, and considerably stretched. The number of discs visible in the proximal portion of the Y in figure 4, lower right, is five. Because this number of discs was observed more than once, this suggests that the element is completely represented in this figure. In other figures no discs at all could be seen, the length and the width of this element being variable depending upon the degree of its extension. The best estimate is that the cytologically visible length of the proximal section of the left arm of the Y chromosome is some 2 to 3 percent of that of the X or the IIR chromosomes in the same cells. (These latter chromosomes are about equal in length.) It may be concluded that the distal and proximal portions of the left arm of the Y chromosome are alike in cytological appearance, the main feature of which is the extreme paleness.

As indicated above, the length of the distal portion of the left arm of the Y is about 65–75 percent of that of the X. The proximal part of the Y amounts to 2–3 similar units. Thus the entire left arm of the Y is 67–78 percent of the X. The right arm of the Y amounts to 4–5 percent of the length of the X according to both SIROTINA's and our own measurements. The entire Y chromosome is, therefore, 71–83 percent as long as the X in the salivary gland cells.

As seen in the metaphase plates of ganglion cells, the ratio of the lengths of the X and Y chromosomes of *D. buscki* is approximately 1 : 0.85. It follows that the length ratios are very nearly the same in the metaphase plates and in the salivary gland cells. This fact is very interesting, because of its bearing on the nature of the Y chromosome. Up to the present our ideas about the Y chromosome of Drosophila have been based almost entirely on the conditions of this element found in other species, particularly in *D. melanogaster*.

The Y chromosome of *D. melanogaster* was identified in the salivary gland cells by PROKOFIEVA (1935). It appears as a small body which contains 8–10 pale discs. BAUER (1936) observed the Y chromosome of *D. pseudoobscura* to be a small bundle of chromonemata with chromioles. He was unable to identify the Y chromosome in either *D. funebris* or *D. hydei*. The Y chromosome of *D. miranda* consists of many euchromatic sections which are imbedded in the chromocenter. MACKNIGHT's (1939) investigations have proved that these sections are the remains of the third chromosome, which have become involved in the sex-determining mechanism. According to PAVAN (1946), the Y of *D. nebulosa* is represented in the salivaries by a small heterochromatic mass. This is all that has been known up to now about Y chromosomes in salivary gland cells of different species of Drosophila. In each of these cases the non-correspondence between the appearance of the Y in the ganglion and salivary gland cells is characteristic.

MULLER and GERSHENSON (1935), MULLER, RAFFEL, GERSHENSON and PROKOFIEVA (1937), and PROKOFIEVA (1937) examined the proximal heterochromatic part of the X chromosome of *D. melanogaster*, which is homologous to the Y chromosome. They put forward two alternative views on the structure of the Y chromosome: (1) The metaphase Y chromosome contains heterochromatic " blocks " of different size, between which genetically active loci are inserted. Due to the invisibility of the heterochromatic blocks in the salivary gland cells, only a small segment which represents the entire genetically active material of the Y chromosome can be detected. (2) Heterochromatic chromosome sections are unspiralized at metaphase, and therefore the Y is represented in the salivary gland cells by a very short element.

There is no compelling reason to assume that the entire Y chromosome and the heterochromatic parts of the X chromosome are alike in structure. This seems not to be the case in *D. buscki*. In this species the relative lengths of the X and Y chromosomes are very similar in metaphases of ganglion cells and in the so-called prophase chromosomes of salivary gland cells. The Y chromosome in the salivary gland cells has a structure on the whole resembling that of the other chromosomes, although it differs from them very strikingly in stainability.

Just why *D. buscki* is the only species known to date in which the heterochromatic portion of the Y is observed to be of such structure cannot be explained with assurance. One of the possibilities is that the appearance of the heterochromatin in the salivary gland cells is due to environmental conditions. In the writer's experience there was one instance when a particular batch of the culture medium proved especially favorable for the observation of the Y chromosomes. Regardless of the strain of mutant Curled-Y the salivary glands of the larvae fed on that medium were uncommonly large and stained very well in all these larvae. The Y chromosome was observed very frequently, though with varying degrees of clarity. Efforts were later made to reproduce culture media of the same quality, but so far without success. Except for the larvae grown on the special medium, the Y chromosome is always a very pale element, and is usually barely visible or even not visible at all.

However it must be noted that, regardless of its poor stainability, the Y chromosome of *D. buscki* has the same structure as the euchromatic elements. Although the discs in the Y chromosome are pale and visible only with difficulty, this chromosome has the appearance of the classical salivary gland chromosomes. About five stainable discs can be identified in the proximal part of the left arm. The number of discs in the distal part of the left arm is not easy to count in the translocation. This part is rarely observed with sufficient clarity along its entire length, and it seldom lies sufficiently flat in preparations to be photographed in a single optical plane. After much labor of counting the discs in different parts of this element, their number was finally estimated to be about 65–70. Adding the discs in the proximal part, the entire heterochromatic arm of Y has about 70–75 discs.

The presence in a chromosome of stainable discs in the salivary gland cells is usually taken as a sign of genetic activity, while unbanded heterochromatin has sometimes been described as genetically "inert." The heterochromatic portion of the Y of *D. buscki*, despite its "euchromatic" appearance, is genetically of so little importance that even in the absence of a large part of it the male zygote may still develop into a more or less normal appearing fly.

SUMMARY

Five X-ray induced dominant mutations have been found which are transmitted from father to son. These mutations carry translocations involving exchanges of sections between the Y chromosome on the one hand and the second or the third chromosome on the other. An analysis of these mutations has permitted the following conclusions to be reached regarding the structure and function of the Y chromosome of *Drosophila buscki*. (1) In metaphase plates of larval ganglion cells the Y chromosome appears as a rod with a knob-like proximal end. The long rod-like part is the left arm, and the knob-like part is the right arm of the chromosome. (2) In salivary gland cells the right arm is represented by a small euchromatic element, whereas the left arm appears as a much longer element which, however, stains very pale. (3) Despite its deficient staining capacity, the left arm shows the classical structure of euchromatic salivary gland chromosomes, that is, an alternation of dark and light discs or bands. The number of discs is estimated to be at least 70–75. (4) The left arm of the Y chromosome seems to be of relatively little importance in development. Males in which most of this arm is absent are viable although sterile. Males which lack approximately the distal ¼ of the left arm are viable as well as fertile. (5) The right arm is apparently more important in development. Aneuploid males lacking most of this arm are inviable. Males which lack the distal ¾ of the right arm are viable but sterile. (6) The centromere is localized between the two arms, possibly at the very boundary of the heterochromatic and euchromatic parts. (7) A suggestion is made regarding the homology of the right arms of the X and Y chromosomes. In this connection, a hypothesis is put forward concerning the origin of XR and YR in *D. buscki* from free microchromosomes such as other species possess, but which are absent in *D. buscki*.

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THE RELATION BETWEEN LIGHT VARIEGATED AND MEDIUM VARIEGATED PERICARP IN MAIZE[1]

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THE present report is based upon a restudy of variegated pericarp in maize, a mutable character which is well known through the important pioneer work of EMERSON (1914, 1917, 1929). The current investigation is concerned primarily with the relation between two distinct phenotypes, light variegated and medium variegated, both of which are unstable. The results obtained, however, bear directly on the allied problem of the kind of change which occurs when variegated is transformed to the stable self colored type.

Two facts emerging from EMERSON'S experiments should be noted here. EMERSON (1917) found that in families obtained by selfing variegated plants heterozygous for the stable red cobbed white allele (WR),[3] variegated (VV) mutates somatically to self colored (RR) more than twice as frequently in heterozygotes (VV/WR), in which it is present once, as in homozygotes (VV/VV) which are duplex for the mutable gene. This observation, which will not be discussed further at present, has been amply confirmed in our work. The second fact was rather startling since, as EMERSON (1929) recognized, it might have been interpreted as evidence of gene contamination. The capacity of a WR allele of given origin seemingly to " stimulate " the mutation of variegated to self red in VV/WR heterozygotes was found to be reduced if, in the preceding generation, the gene passed through a VV/WR plant which showed a low grade of variegation. In a gross statistical sense, this relation also is confirmed in our experiments.

EMERSON (1929) considered it unlikely, however, that the WR allele itself was the element affecting the VV/WR heterozygote. He assumed, on the other hand, that the mutation modifier present in WR stocks was a gene (or genes) closely linked to, but distinct from, the P locus and subject to crossing over with alternative forms of the modifier occurring in the variegated races tested. Definitive evidence for the postulated second locus was not brought forward. The additional facts now at hand make it improbable that a satis-

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[3] The alleles occurring at the P locus are referred to throughout the paper by the superscripts of the symbols only. The first letter in the superscript indicates pericarp color and the second, cob color. The combined pericarp-cob characters are transmitted in unitary fashion. The full symbols are P^{VV}, variegated pericarp and cob; P^{RR}, red pericarp and cob; P^{WR}, white (colorless, or nearly colorless) pericarp, red cob; P^{WW}, colorless pericarp, white cob (ANDERSON 1924).

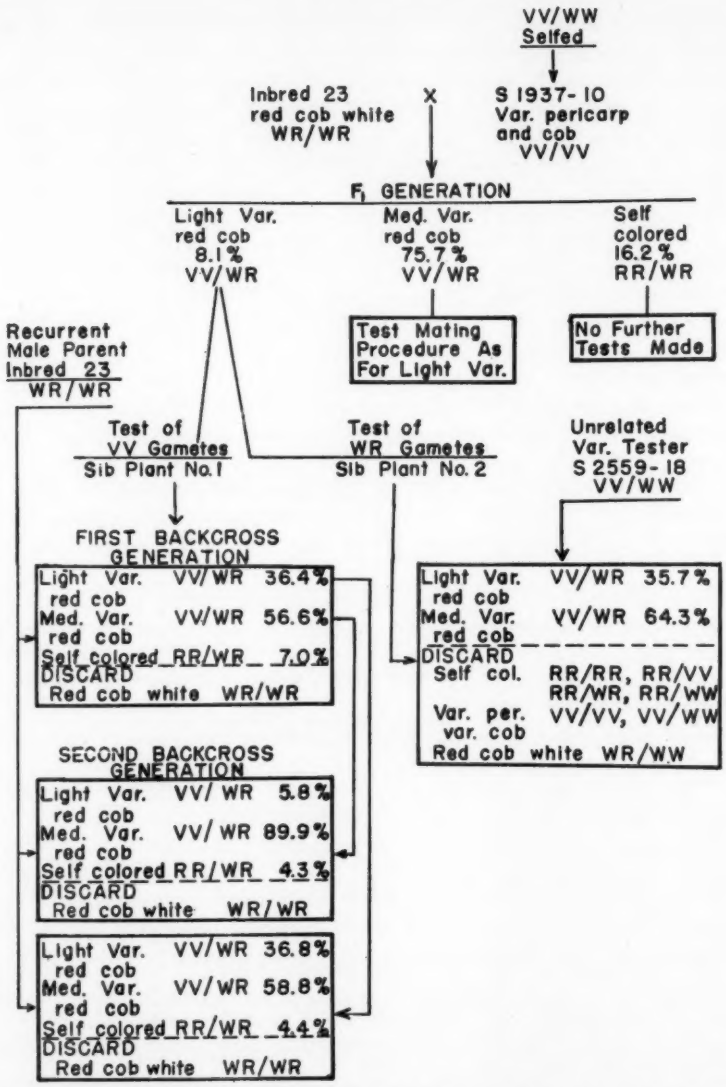


FIGURE 1.—The mating plan followed in testing the composition of the *VV* and *WR* gametes from *VV/WR* heterozygotes with reference to factors conditioning light and medium variegation. The numerical results presented for illustrative purposes relate to certain families involving inbred line 23.

factory explanation of the phenomenon can be formulated in these classical genetic terms.

Two further points should be mentioned bearing on the relation between the current work and that on variegated pericarp which preceded it. Stocks were employed in the present study in which two classes of variegated plants, light and medium, could be distinguished with much greater regularity than appears to have been possible with EMERSON's material. Variegated pericarp in maize is subject to wide variation in phenotypic expression, and the use of families comprising sharply distinct classes of variegated plants is an important methodological advantage. The second consideration is the weight to be given the occurrence of twin spots on variegated ears. Twin spots (e.g., a light variegated area adjacent to a self colored patch on an otherwise medium variegated ear) of one or another kind have been recognized since the beginning of genetic studies on maize pericarp. They occur sporadically, however, and, when present, are often of insufficient size and contrast to be scored with certainty. It has not been clear from the previous work whether twin spots are of basic significance for variegation or are merely an interesting minor class of pericarp mutations. EMERSON did not make a point of them. We regard the evidence from twin spots as fundamental to an understanding of the central problem.

MATERIALS AND EXPERIMENTAL PROCEDURE

EMERSON's work suggested that the relationship most in need of clarification was the apparent transmission by both *VV* and *WR* gametes from *VV/WR* heterozygotes of major genetic elements affecting the variegated phenotype. Of particular interest was his observation that the capacity of the *WR* gamete to condition the expression of variegated seemed to be influenced by the variegation grade of the parent plant. A two-fold body of evidence was needed, one bearing on the composition of the *VV* gametes and the other on the properties of the *WR* gametes. It appeared important also that the two sets of facts be obtained under conditions in which they could be related to each other directly. The rather simple mating system used in the present experiments, illustrated diagrammatically in figure 1, met these requirements.

A series of *VV/WR* and *VV/WW* hybrids were established by pollinating a number of inbred field corn lines with a single collection of pollen from a homozygous variegated plant both of whose *VV* genes had been derived from a selfed *VV/WW* parent. It was a very helpful circumstance that each of the resulting groups of hybrids comprised two discrete classes of individuals, light variegated and medium variegated, rather than a population varying continuously in variegation grade, as had been expected. These light and medium variegated F_1 hybrids were then tested in two ways.

One set of plants in each hybrid series was mated recurrently with the appropriate *WR/WR* or *WW/WW* inbred line through two generations. This procedure was designed to disclose the properties of the *VV* gametes emerging from the hybrids.

The *WR* gametes from the two classes of variegated F_1 hybrids were separately assayed for their influence on variegation. Single collections of pollen from unrelated *VV/WW* individuals were spread widely over the silks of a parallel set of the hybrid plants in question. A series of *VV/WR* offspring, recognized by their red cob, was thus obtained in which the *WR* gene marked a sample of gametes of known origin in terms of the variegation class, light or medium, of the parent plants. This kind of test mating served not only to extract *WR* from the two types of hybrids but also to reestablish directly *VV/WR* combinations in which the possible effects on variegation of the inheritance accompanying the *WR* allele could be observed.

A single collection of pollen from plant S1937-10, which bore a light variegated ear, was the source of the variegated allele introduced into all the F_1 hybrids with one unimportant exception. The ancestry of S1937 traced to a previously unpedigreed variegated pericarp plant whose pollen was used on a white cobbed white inbred line known as 4Co63. One of the resulting hybrids was then backcrossed to 4Co63, giving rise to family S1707. Plant 1 in the latter family was selfed, and from the seed obtained duplicate families, S1930 and S1755, were grown in different years. The immediate parent of S1937 was plant S1755-3, selfed. The single exception to the use of S1937-10 as the foundation variegated plant was the use of seed from a cross with S1930-1 as the pollen source in obtaining the line 8 hybrids referred to in table 5. S1930 and S1937 had a common ancestor in plant S1707-1, selfed. There was no difference in the results obtained with the two close relatives.

The inbred field corn lines used were: 8, 22, 23, 28, 70, M14, 40B, and the aforementioned 4Co63. The first six of these are red cobbed whites; the last two are white cobbed whites. All had been selfed 10 generations or more, and were highly uniform. The lines are unrelated to each other, and are representative of the inbred stocks currently in use in the production of commercial hybrid field corns adapted to southern Wisconsin. Strains 8, 22, 23, 28, and 70, being of Wisconsin origin, are commonly referred to in the hybrid corn literature as W8, W22, etc. The W prefix is omitted here in order to avoid possible confusion with the same symbol used in designating certain *P* alleles. The expanded symbols *WR*-8, *WR*-22, etc., appearing occasionally in the paper, merely indicate the inbred line from which the *WR* allele in question was derived. Generally speaking, the red cobbed whites of different origins are not distinguishable phenotypically from each other.

Variegated plants descended from an open pollinated stock obtained from a Colorado farmer and quite unrelated to S1937 were employed as pollen parents in the test crosses referred to in table 5. Families S2559 and S2560 were obtained by selfing two plants grown from seeds on the same ear. Family 7-5 was similarly obtained from another ear.

A definitive classification of the mutations of variegated to self color would require an accounting of each red area appearing on a kernel. Such a classification is not possible. Only the larger stripes and patches can be certainly

identified and counted. Many small colored areas are discrete, but they grade downward in size until the mutant phenotype is represented by rather weakly pigmented tissue within which stripes cannot be distinguished even with magnification. Size of the well-defined colored areas is an index of the number of cell divisions which have intervened between the initial mutated cell and maturity of the tissue. Apparently mutations to red pericarp may occur at any stage of ontogeny although, as ANDERSON and EYSTER (1928), have shown, they are much more frequent, relative to cell number, at the later stages of development. These characteristics of variegated pericarp make it necessary to use purely empirical methods in estimating the frequency of mutations over the entire spectrum as, for example, throughout a single ear. What is scored in such a case is a generalized phenotype whose characteristics are determined by the number and distribution within a complex cell lineage of numerous mutations of the same kind.

After experimenting with other methods, a procedure was adopted for classifying entire variegated ears similar to that used by EMERSON (1929). Ears were selected as standards representing seven classes varying in intensity of striping from very light, i.e., one stripe or less per kernel, on the average (class 1), to very heavy striping (class 7). Each test ear was compared with the standards and then assigned the variegation grade of the class to which it corresponded. The method is rapid and, as EMERSON found, gives repeatable results for which, however, high precision cannot be claimed. Occasionally families are encountered which cannot be classified satisfactorily by this procedure due to nonconformity of the ears in one or another respect to the standards chosen. This shortcoming is disconcerting in particular instances, but it was found not to detract seriously from the general usefulness of the method.

A much simpler method of classifying our material came to hand with recognition of the fact that the variegated plants in the F_1 and backcross families usually fell into two discrete classes. These classes were termed light variegated and medium variegated, respectively. They are illustrated in figure 2, together with the stable self colored type to which both mutate. This dichotomy within families segregating variegated was not perceived until the group involving inbred 4Co63, on which figure 2 is based, was classified. Once the difference was recognized in the 4Co63 families, in which it was sharply expressed, it was looked for and found at once in the others.

Generally speaking, light variegated ears were found to fall into classes 1 and 2 and medium variegated ears into classes 3 and 4, rarely 5, as these categories were delimited by the standard ears referred to earlier. The respective light and medium variegated phenotypes were found to be fairly constant for related families, but they varied somewhat from one inbred group to another, depending presumably on the residual inheritance.

The validity of the classification into light variegated and medium variegated depends upon the regularity of occurrence of a discontinuity between

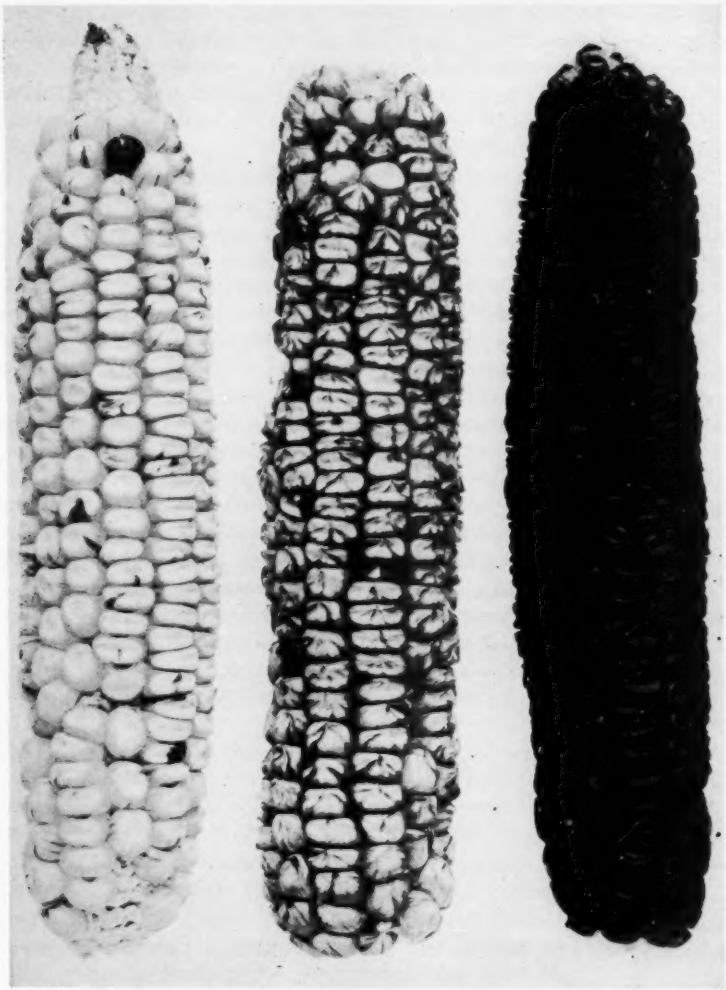


FIGURE 2.—Light variegated (left), medium variegated (middle), and self colored (right) ears in the 4Co63 backcross series.

these classes in segregating families. A discontinuity was clearly apparent, with only a few exceptions, in both the first and second backcross generation families involving inbreds 8, 22, 23, and 4Co63. The same statement applies to the test cross families listed in table 5, involving inbreds 8, 22, 23, 28, and 70. When present at all, intermediate ears were few in numbers, and the remaining ears corresponded closely to the light and medium variegateds,

respectively, in sib families in which the distribution definitely was discontinuous. A few " bizarre " ears were encountered in which there appeared to be an irregular mixture of more or less well defined light and medium variegated areas. Such ears were placed according to the class of tissue which predominated. Some of the various exceptional ears among the several thousand scored may have represented genetically different variegation types. They were not hand-pollinated and so could not be tested further. Others were probably merely variant members of the two prevailing classes.

In contrast to the above groups, several of the backcross families based upon inbreds M14 and 40B were found difficult or impossible to classify in these terms. This was especially the case in the second backcross generation which was grown in 1951, an unusually cool season throughout, in which a killing frost occurred before the ears in these relatively late maturing families were fully ripe. Since pericarp color attains its full development late in growth of the plant, immaturity is a handicap in classification. The ears in more than half these families, however, could be separated clearly into the two phenotypes. This fact suggests that the M14 and 40B series do not differ basically from the others. The residual inheritance carried by these two inbreds, including that for late maturity, appears to be relatively unfavorable, however, for sharp expression of the difference between light and medium variegated. The available data on the M14 and 40B series are not reported because of their varying dependability from family to family. These stocks are being further studied.

EXPERIMENTAL DATA

The data bearing on the distribution of variegated and self colored in the first and second backcross families have been tabulated according to the respective inbred lines which served as the recurrent parents. Since a common variegated parent, S1937-10, was involved in all the initial matings, the four series of families, corresponding to inbreds 8, 22, 23, and 4Co63, respectively, might be expected to be similar. It will be seen that, in fact, the same general relations are in evidence in each backcross group. There are significant variations in detail, however, both between and within groups.

F_1 generation

Three kinds of offspring, light variegated, medium variegated, and self colored, occurred in approximately the same proportions in each of the F_1 families resulting from application of a single collection of pollen from the homozygous variegated plant, S1937-10, to inbred 8, 22, 23, 28, and 4Co63. The distribution among the three phenotypes of 279 individuals classified in the five families was 10.7 percent, 72.4 percent, and 16.8 percent, respectively.

Backcrosses to inbred 8

The data relating to the first and second backcross generations of the appropriate F_1 plants to inbred 8 are brought together in table 1. The variegation

TABLE 1

Distribution of offspring of VV/WR-8 light and medium variegated plants backcrossed once and twice to inbred line 8.

Family	Var. grade seed parent	Offspring		% among colored		
		Total	Colored	Light var.	Med var.	Self
		First backcross generation				
6-70	1	116	51	41.2	52.9	5.9
6-71	2	96	48	52.1	45.8	2.1
6-72	2	188	98	52.0	44.9	3.1
6-73	2	185	89	42.7	53.9	3.4
6-74	3	336	175	4.0	91.4	4.6
6-75	3	283	159	5.7	88.6	5.7
6-77	4	272	141	1.4	95.1	3.5
		Second backcross generation				
62-1	1	187	85	65.9	31.7	2.4
62-2	2	186	105	54.3	41.9	3.8
62-3	1	119	59	49.2	45.7	5.1
62-4	1	94	45	57.8	37.8	4.4
62-5	2	117	55	49.1	45.4	5.5
62-6	2	158	84	51.2	45.2	3.6
62-7	2	127	59	47.5	50.8	1.7
62-8	1	90	45	42.2	51.1	6.7
62-9	3	95	45	11.2	86.6	2.2
62-10	3	113	71	5.6	93.0	1.4
62-11	3	153	78	5.1	83.4	11.5
62-12	3	111	54	3.7	90.7	5.6
62-13	3	158	70	5.7	85.7	8.6
62-14	3	198	107	6.5	87.0	6.5
62-15	3	128	73	1.4	90.4	8.2
62-16	3	114	57	1.8	96.4	1.8
62-17	3	215	107	1.9	93.4	4.7
62-18	3	158	81	2.5	93.8	3.7
62-19	3	216	109	9.2	88.0	2.8
62-20	3	170	78	5.1	88.5	6.4
62-21	3	142	78	1.3	94.9	3.8

grade of the parent ear from which each family was derived is shown in column 2. The pistillate parents in both generations were heterozygous for colorless pericarp, so that about one-half the offspring are of the latter type. These individuals are of no immediate interest except as they confirm the fact that the distribution of colored (variegated plus self red) and colorless pericarp is Mendelian.

Five characteristics of the data in table 1 are noteworthy.

1. The colored pericarp plants in each family include the three phenotypes which appeared in F_1, namely, light variegated, medium variegated, and self colored.
2. Two distinct kinds of families occur with reference to the frequencies of light and medium variegated individuals; the proportion of light

variegated plants is high in one group and low in the other, as shown by the values in the fifth column.

3. The families containing a high proportion of light variegated plants were derived from light variegated ears (class 1 or 2) and those with few such individuals arose from medium variegated ears (class 3 or 4).
4. The frequency of occurrence of self colored ears is of the same order as that of light variegateds among the offspring of medium variegateds.
5. The results in the first and second backcross generations are alike.

The similarity in breeding behavior of medium variegateds descended from light and medium variegateds, respectively, is illustrated by the data from families 62-9 to 62-21 in table 1. Each of these 13 twice backcrossed families was grown from the seeds on a medium variegated (class 3) ear. All were descended from a single F_1 hybrid family, 6-1, containing both light variegated and medium variegated plants. Families 62-9 and 62-10 were descended from a light variegated (class 1) ear, families 62-11 to 62-15 from a medium variegated (class 3) ear, and families 62-16 to 62-21 from a medium variegated ear of class 4, in the F_1 generation. It will be noted that the three groups of twice backcrossed families are similar in the proportions of light variegated,

TABLE 2

Distribution of offspring of VV/WR-22 light and medium variegated plants backcrossed once and twice to inbred line 22.

Family	Var. grade seed parent	Offspring		% among colored		
		Total	Colored	Light var.	Med var.	Self
First backcross generation						
6-80	1	181	85	97.6	0	2.4
6-81	1	200	98	63.3	34.7	2.0
6-82	2	286	142	12.7	84.5	2.8
6-83	4	240	130	1.5	93.1	5.4
6-84	4	313	157	0.6	96.2	3.2
6-85	4	195	94	2.1	91.5	6.4
6-86	4	340	167	2.4	94.0	3.6
Second backcross generation						
62-22	2	200	102	69.6	21.6	8.8
62-23	2	113	53	54.7	43.4	1.9
62-24	2	131	72	79.2	16.6	4.2
62-25	2	150	77	55.8	37.7	6.5
62-26	2	87	44	79.5	18.2	2.3
62-27	3	168	91	36.3	62.6	1.1
62-28	3	159	77	2.6	93.5	3.9
62-29	3	149	80	2.5	95.0	2.5
62-30	3	134	74	12.2	85.1	2.7
62-31	3	173	81	2.5	92.6	4.9
62-32	3	157	75	21.3	78.7	0.0
62-33	3	109	56	3.6	92.8	3.6
62-34	3	247	126	2.4	92.8	4.8

medium variegated, and self colored ears which they contain. This means that medium variegated plants of the same kind as those produced directly by medium variegated parents may be extracted from light variegated individuals.

Unfortunately, corresponding data bearing on the comparability of light variegated plants derived from light and medium variegated parents, respectively, are not available. This gap in the evidence is due to failure in the first backcross generation of numerous plants to form ears usable for seed as a result of severe insect injury. Evidence of another kind, however, presented in table 5, which will be considered later, indicated that light variegateds originating in these two ways are probably alike.

Backcrosses to inbred 22

The first and second backcross generation data from hybrids between inbred line 22 and S1937-10 are brought together in table 2. Again, two distinct kinds of families, distinguishable on the basis of the proportion of light variegated plants which they contain, are represented. There is much more heterogeneity between families within each of the two main groups, however, than was found in the backcrosses to line 8 discussed above. Family 6-80 in the first backcross generation, for example, comprised 83 light variegated, no medium variegated, and two self colored ears. That is to say, 97.6 percent of the colored offspring were light variegated. One sister family, 6-81, contained 63.3 percent light variegateds, and a second sister family, 6-82, only 12.7 percent. All three of these families were based on light variegated (class 1 or 2) seed ears. It is evident, however, that in spite of this wide variation between families, the group as a whole differs sharply from the families derived from medium variegated (class 4) sister ears. Among the latter progenies, the proportion of light variegated plants in no case exceeds 2.4 percent.

The data from the second backcross generation are obviously heterogeneous also. Here too, however, the frequencies of light variegated plants from the light and medium variegated parent ears, respectively, do not overlap, although the results from family 62-27 raise some doubt on this point. The 62-27 parent seed ear was scored as grade 3 variegated, a classification which has been confirmed by reexamination of the remnant. The progeny contained 36.3 percent light variegated individuals, a value approximately midway between the average of the other families from class 3 ears and the average of families from the light variegated (class 2) parent ears. Furthermore, it will be noted that among the families based on class 3 ears, excluding 62-27, the proportion of light variegated offspring varies from 2.4 percent to 21.3 percent. The possible causes of these variations will be considered later.

Backcrosses to inbred 23

The third body of data bearing on the composition of the *VV* gametes formed by *VV/WR* heterozygotes is summarized in table 3. The initial cross in this case involved S1937-10 and inbred line 23. Two successive backcrosses to the red cobbed white inbred were made in accordance with the general plan.

TABLE 3

Distribution of offspring of VV/WR-23 light and medium variegated plants backcrossed once and twice to inbred line 23.

Family	Var. grade seed parent	Offspring		% among colored		
		Total	Colored	Light var.	Med. var.	Self
First backcross generation						
6-89	2	194	100	44.0	53.0	3.0
6-90	2	267	129	36.4	56.6	7.0
6-91	2	245	134	38.1	56.7	5.2
6-92	3	274	139	5.0	82.8	12.2
6-93	3	237	124	5.6	80.7	13.7
6-94	4	218	101	6.9	84.2	8.9
6-95	4	177	93	6.5	84.9	8.6
Second backcross generation						
62-43	2	135	68	36.8	58.8	4.4
62-44	2	142	60	40.0	53.3	6.7
62-45	2	225	103	48.5	42.8	8.7
62-46	4	132	69	5.8	89.9	4.3
62-47	4	90	44	11.4	77.2	11.4
62-48	4	142	72	5.6	76.3	18.1

The results are closely parallel to those obtained from the test matings involving inbred line 8, except in one respect. Whereas approximately 50 percent of the offspring from the light variegated seed ears (classes 1 or 2) were light variegated in the formed case, the average proportion of plants of this kind in both the first and second generation backcross families involving line 23 are closer to 40 percent. Contrariwise, the medium variegated ears in the line 23 group yielded families containing a higher frequency of light variegated plants than did the corresponding class of ears in the line 8 families. Since the differences mentioned occur rather regularly, and the respective bodies of data on which they are based are relatively homogeneous in contrast to those obtained from the line 22 backcrosses, the disparities in proportion of light variegated plants may be presumed to have a genetic basis. Aside from this point, the data from the line 23 backcrosses are in remarkably close accord with those from the line 8 test matings. Thus, the five basic characteristics of the backcross families to which attention was called on reviewing the results with line 8 are again clearly in evidence.

Backcrosses to inbred 4Co63

The white cobbed colorless pericarp inbred line 4Co63 was the recurrent parent in the backcrosses on which table 4 is based. The results given in the table are in conformity with those reported for the matings involving inbreds 8, 22, and 23 in showing that two categories of families occur, one with a high proportion of light variegated individuals and the other containing few plants of this class. The former type of families is derived from light variegated seed

ears and the latter exclusively from medium variegated parent ears. The variation in proportion of light variegated plants between families within each of these categories, however, is relatively high.

Evidently homozygosity for the residual inheritance does not stabilize the proportion of light variegated plants appearing in the separate families within each of the two recognized types. The data in table 4 support this conclusion. The 4Co63 families were the most highly inbred among the four backcrossed series. This resulted from the fact that the foundation variegated plant, S1937-10, used in the original outcrosses to the several inbreds, derived 75 percent of its inheritance from 4Co63. The two successive matings subsequently

TABLE 4

Distribution of offspring of VV/WW-4Co63 light and medium variegated plants backcrossed once and twice to inbred line 4Co63.

Family	Var. grade seed parent	Offspring		% among colored		
		Total	Colored	Light var.	Med. var.	Self
		First backcross generation				
6-109	2	69	33	78.8	18.2	3.0
6-110	2	93	49	51.0	47.0	2.0
6-111	4	95	47	12.8	85.1	2.1
6-112	4	122	64	1.6	87.5	10.9
6-113	4	159	86	9.3	84.9	5.8
6-114	4	149	77	10.4	85.7	3.9
6-115	4	160	82	6.1	85.4	8.5
		Second backcross generation				
62-35	1	53	28	89.3	10.7	0.0
62-36	4	146	62	6.5	79.0	14.5
62-37	4	118	60	10.0	86.7	3.3
62-38	4	131	64	6.2	79.7	14.1
62-39	3	81	39	7.7	89.7	2.6
62-40	4	197	103	7.8	78.6	13.6
62-41	3	81	39	12.8	79.5	7.7
62-42	4	131	61	3.3	90.1	6.6

made to this inbred line would result in a second backcross generation almost 95 percent of whose inheritance coincided with that of 4Co63. In spite of the high degree of convergence of the 4Co63 test families toward the recurrent 4Co63 parent inbred line, a considerable amount of heterogeneity remains in the families arising from ears of a given variegation class.

A few families have been grown from backcrossed self colored ears in each of the above four series. They have regularly shown equal proportions of self colored and colorless offspring, within the limits of random sampling.

The proportion of light variegated and self red offspring of medium variegated plants

A summary is given in table 6 of the frequencies with which light variegated and self red plants occurred in the families from medium variegated seed ears

TABLE 5

VV/WR segregates from light and medium variegated VV/WR F_1 plants pollinated by VV/WW.

Family	Seed Parent		VV/WW pollen parent	Poll. date	Variegated offspring					
					Light		Medium		Total	% light
	Genotype	Var. grade			No.	Mean grade	No.	Mean grade		
N156	VV/WR-8	1	7-5-106	7-22	26	1.61	14	3.14	40	65.0
N161	"	4	"	"	7	1.42	46	3.13	53	13.2
N164	"	1	7-5-106	7-23	27	1.37	19	3.21	46	58.7
N165	"	1	"	"	21	1.61	14	3.07	35	60.0
N168	"	4	"	"	3	1.33	46	3.34	49	6.1
N169	"	5	"	"	4	1.50	36	3.22	40	10.0
N30	VV/WR-22	2	S2559-16	7-28	35	1.48	23	3.04	58	60.3
N36	"	4	"	"	9	1.44	41	3.12	50	18.0
N49	VV/WR-22	2	S2559-16	7-27	15	1.40	5	3.00	20	75.0
N50	"	2	"	"	17	1.35	14	3.00	31	54.8
N53	"	4	"	"	19	1.99	25	3.08	44	43.2
N54	"	4	"	"	8	1.50	27	3.07	35	22.9
N58	VV/WR-23	1	S2559-18	7-23	10	2.00	18	3.16	28	35.7
N62	"	4	"	"	0	....	16	3.25	16	0.0
N63	"	5	"	"	2	2.00	32	3.21	34	5.9
N64	VV/WR-23	2	S2559-18	7-24	5	2.00	36	3.06	41	12.2
N65	"	2	"	"	28	1.89	29	3.03	57	49.1
N68	"	4	"	"	6	1.83	43	3.07	49	12.2
N69	"	4	"	"	6	1.83	51	3.11	57	10.5
N70	"	4	"	"	6	1.66	46	3.03	52	11.5
N72	VV/WR-23	2	S2559-18	7-25	16	1.75	3	3.00	19	84.2
N76	"	4	"	"	14	1.58	33	3.00	47	29.8
N77	"	4	"	"	6	1.66	29	3.06	35	17.1
N85	VV/WR-28	1	S2559-19	7-23	8	1.62	35	3.02	43	18.6
N90	"	4	"	"	5	1.60	41	3.04	46	10.9
N91	"	4	"	"	3	1.66	33	3.15	36	8.3
N92	VV/WR-28	1	S2559-19	7-24	8	1.75	40	3.02	48	16.7
N93	"	2	"	"	6	1.16	22	3.04	28	21.4
N97	"	3	"	"	2	2.00	21	3.10	23	8.7
N98	"	4	"	"	5	1.60	53	3.09	58	8.6
N118	VV/WR-70	1	S2560-5	7-23	18	1.94	14	3.57	32	56.2
N121	"	3	"	"	4	1.75	51	3.02	55	7.3
N123	"	4	"	"	4	1.50	28	3.53	32	12.5
N124	VV/WR-70	1	S2560-5	7-24	17	1.70	13	3.38	30	56.7
N125	"	1	"	"	10	1.60	25	3.28	35	28.6
N127	"	1	"	"	4	2.00	18	3.44	22	18.2
N130	"	3	"	"	4	1.75	31	3.64	35	11.4

in the four backcross series. The proportion of self reds to light variegateds in the total is 260 to 206, or a ratio of 1.3 : 1. The individual comparisons which may be made in the table disclose substantial variations in the ratio between both series and backcross generations, for which no explanation is at hand. Certain regularities, however, are in evidence. The agreement in the ratios between generation, for example, is relatively close in the case of the 8 and 23 backcross series. This fact is in accord with the comparative uni-

formity in behavior of these two groups in other respects also, including the percentages of light variegated offspring from family to family obtained from the light variegated seed ears. These ratios are of interest when considered in relation to the evidence from twin spots, to be presented later, which shows that mutations of medium variegated to light variegated and self color often occur concurrently.

Influence of WR gametes extracted from light and medium variegated VV/WR heterozygotes on the phenotypic expression of variegated in the next generation

The results of the experiments made to disclose the composition of *WR* gametes from light and medium variegated heterozygotes (*VV/WR*) with reference to factors conditioning variegated pericarp are assembled in table 5. All the hybrids tested in this series, except one, had plant S1937-10 as their variegated parent. A closely related variegated individual, S1930-1, was used in the cross with inbred 8. This series of F_1's is directly comparable, therefore, to the *VV/WR* hybrids whose *VV* offspring are recorded in tables 1 to 4 in so far as the inbred parent lines are the same. The 4Co63 inbred line was omitted from the present tests because its white cob made it unsuitable for the kind of testcross made; and two red cobbed inbred lines, 28 and 70, not used in the other experiments, were added.

A single type of test mating was employed. A given collection of pollen from an unrelated variegated plant heterozygous for white cob, colorless pericarp was distributed over the silks of a sufficiently large number of F_1 *VV/WR* sibs to include, in the cases reported, both light and medium variegated plants. Thus, *WR* gametes from otherwise closely comparable light and medium variegated plants were brought into association with a common array of *VV*

TABLE 6

Proportion of light variegated and self red offspring in families derived from medium variegated backcrossed ears.

Backcross series	No. of families	Number of plants		Ratio
		Light var.	Self red	R/V
First backcross generation				
8	3	18	22	1.2
22	4	9	24	2.7
23	4	27	51	1.9
4Co63	5	28	23	.8
Totals:	16	82	120	1.5
Second backcross generation				
8	13	43	58	1.3
22	7	36	19	0.5
23	3	13	21	1.6
4Co63	7	32	42	1.3
Totals:	30	124	140	1.1

gametes. The relevant class of offspring bore red cobbed variegated ears, and only this class was scored.

Sometimes pollen was taken from the same *VV/WW* individual on successive days. The results obtained with each such collection are entered separately in table 5.

It was a matter of great interest to find that the red cobbed variegated ears in the test cross families fell into two distinct groups, light variegated and medium variegated. That is to say, the same two types of variegation which characterized the F_1 pistillate parents from which the *WR* alleles were derived reappeared in the offspring, even though the latter carried *VV* genes from a different source. Table 5 shows the distribution of the ears between the light variegated and medium variegated classes in each family. The classification on this basis was clear cut except in the case of two families among the 37. The discontinuity between light variegated and medium variegated was not sharp in N53 and N127, so that the counts given in these families may err somewhat in either direction.

The ears within the respective main variegation classes in each family were scored also for variegation grade against the standard set of ears. The mean values thus obtained are included in the table.

Eleven groups are available within each of which a comparison may be made between the eventual effect on variegation of *WR* gametes derived, respectively, from light variegated and medium variegated *VV/WR* heterozygotes. The pistillate parents in each group are sibs to which pollen from a single collection were applied.

With due regard to the fact that the relations to which attention is called are much more clearly shown in certain groups than in others, the salient features of the data in table 5 may be summarized as follows:

1. Each of the 37 families, with one exception, which might be due to random sampling, comprises two classes of variegated plants, light and medium.
2. Comparisons on a within group basis show that progenies from light variegated seed ears contain a higher percentage of light variegated plants than progenies from medium variegated seed ears. The one exception is family N64, from a light variegated ear, in which the proportions of light variegated offspring is unaccountably low.
3. In a few of the groups there is a wide gap between the percentages of light variegated offspring from light and medium variegated seed ears, respectively. The two groups involving *VV/WR*-8 hybrids pollinated by plant 7-5-106 are the most clearly differentiated in this respect.
4. Within a group, the mean variegation grade of the light variegated offspring from light variegated seed ears is essentially the same as the mean grade of the same class of plants from medium variegated seed ears. The same relation holds for the medium variegated offspring of the two respective classes of parent ears.

5. Progenies from light and medium variegated ears of comparable parentage thus differ only in the relative percentages of light variegated and medium variegated plants which they contain.

The percentages of light variegated offspring in progenies from seed ears of a given variegation class vary widely from family to family. Possible reasons for the high variability both here and in the backcross series will be considered more fully later in the paper. Meanwhile it may be noted that part of the present variation may have arisen from the variegated plants from which the pollen was taken for the test crosses. At the time the pollinations in question were made it was thought that the test crosses would be sufficient in themselves to provide the evidence needed. Consequently, no matings were made by which the pollen parents could be characterized independently for their content of factors affecting variegation. In spite of the uncertainties thus created regarding the origin of some of the variability present, the evidence as a whole shows unmistakably that *WR* gametes arising from light variegated *VV/WR* heterozygotes, when recombined with *VV* gametes, yield families containing a higher proportion of light variegated plants than similarly tested *WR* gametes produced by otherwise comparable medium variegated plants. This result is comparable, in a broad statistical sense, to the observation which EMERSON (1929) made on the properties of *WR* gametes from variegated heterozygotes of different grades.

When the data in table 5 are considered in relation to the evidence obtained in the backcross series (tables 1 to 4) an additional fact of particular significance becomes apparent, namely, that the direction of the effect associated with variegation grade is the same for both the *WR* gametes and the *VV* gametes arising from a given *VV/WR* heterozygote. If the latter is light variegated, then both the *WR* and *VV* gametes, when appropriately tested, tend to give a high proportion of light variegated offspring. If the heterozygote is medium variegated, both the *WR* and *VV* gametes yield relatively low percentages of light variegated progeny.

Since the differential between light variegated and medium variegated pericarp thus assorts more or less freely with both the *VV* and *WR* alleles, it cannot reside in the *VV* gene and, indeed, must be separate from the *P* locus.

Twin spots

Mutations of variegated pericarp sometimes appear as twin spots. These consist of a self colored area adjacent to a sector showing a lighter grade of variegation than prevails elsewhere on the ear. Twin spots are thus complementary in the sense that one component involves an increase and the other a decrease in pigmentation, relative to the parent phenotype. The more readily identifiable twins vary in size from a single kernel upward. A relatively large one is illustrated in figure 3. The number of twins appears to differ widely between strains. The first estimate of frequency made in our laboratory was based upon a diverse assemblage of 2915 open pollinated variegated ears,

heterozygous for red cobbed white. The individuals bearing self colored patches covering 10 kernels or more and not extending to the butt or tip of the ear were picked out. Within this sample, comprising 57 ears, the self colored mutant patches were found to be twinned in 26 percent of the cases. This observation suggested that twinning probably was an important aspect of the variegation phenomenon, and also raised the hope that twins of adequate size

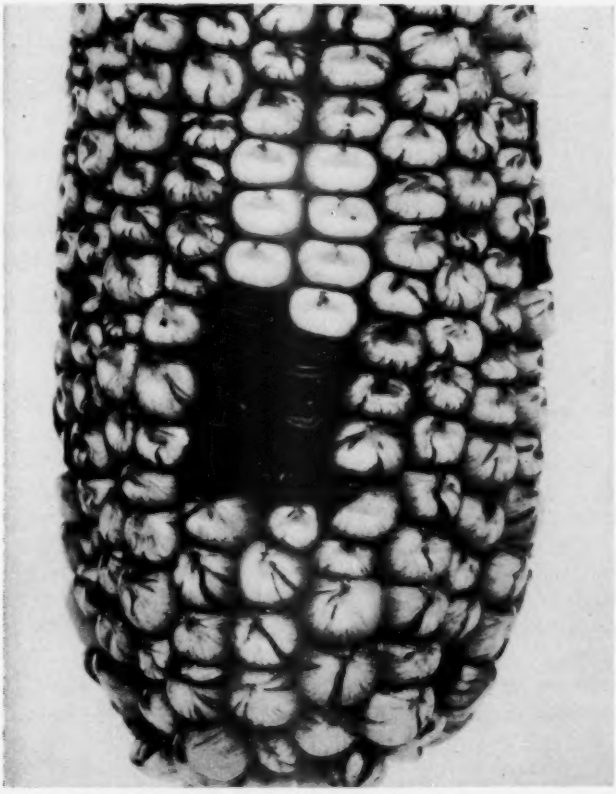


FIGURE 3.—Twin spots, consisting of light variegated and self colored kernels, on a medium variegated ear.

might be encountered frequently enough among suitably bred hand-pollinated ears to enable progeny tests of the three phenotypes concerned to be made.

The frequency of twin spot formation was estimated in 23 of the second generation backcross families listed in tables 1, 3, and 4. The medium variegated ears in these families were sorted for individuals bearing a self colored area covering five kernels or more. This somewhat arbitrary lower limit for size was chosen on the grounds that it probably excluded most of the cases in

which it is difficult to determine satisfactorily whether a given self colored patch is or is not twinned. It will be seen from the data, which are summarized in table 7, that in all three backcross series, the frequency of twinning exceeds 50 percent, and in the case of the 4Co63 group, reaches approximately 90 percent. These values should not be generalized because they are based upon a sample which includes only the few mutations to self color which occurred early enough in ontogeny to give relatively large mutant patches. Since it is evident, however, that many smaller self colored areas are twinned also, even though scoring in this size range is less certain, it can be concluded that a significant proportion of the mutations of variegated result in two complementary phenotypes. Only a beginning has been made in the genetic analysis of such twins. The few data now available are presented in table 8.

Each of the five parent plants represented in table 8 was heterozygous for colorless pericarp, and the pollen applied to them carried colorless pericarp also. One-half of the offspring, within sampling limits, were found to have colorless pericarp, as expected. The remaining data are too few to be conclu-

TABLE 7

Frequency of twin spot formation on medium variegated ears in second generation backcross families involving inbreds 8, 23, and 4Co63.

Backcross group	Number of medium variegated ears			Percent twinned
	Total examined	Bearing 5-kerneled red patches	Twinned	
8	908	57	33	57.9
23	151	20	11	55.0
4Co63	354	28	25	89.3

sive on the point, but they support the view that the light variegated, medium variegated, and self colored sectors agree in genotype with entire ears of the three respective kinds (cf. tables 1 to 4 for progenies from light and medium variegated ears). The colored offspring from self colored kernels are regularly self colored.

Tests are in progress on the inheritance conditioning variegated pericarp transmitted through the *WW* or *WR* gametes from the five groups of plants represented in table 8.

If it is proved eventually that light variegated–self colored twin spots on otherwise medium variegated ears correspond genotypically to the same respective classes of offspring from medium variegated parents, a conclusion to which the present limited evidence points, the fact is highly important. It means that, in medium variegated plants two mutations, one to light variegated and the other to self color, may arise concurrently from the same single event. The fact was long since established by EMERSON that the change of variegated to self color involves a mutation at the *P* locus. The present investigation shows that the differential between light variegated and medium variegated, on the other hand, rests upon a factor at a site distinct from *P* and

TABLE 8

Phenotypes of plants reared from light variegated, medium variegated, and self red kernels from ears bearing twin spots.

Family	Phenotype of parent sector	Colored offspring			Colorless	Total
		Light var.	Med. var.	Self red		
			Ear 1[1]			
63-12	Light var.	1	0	1	2	4
63-13	Med. var.	0	6	0	6	12
63-14	Self red	0	0	2	1	3
			Ear 2[1]			
63-15	Light var.	1	1	1	7	10
63-16	Med. var.	0	6	1	4	11
63-17	Self red	0	0	0	4	4
			Ear 3[1]			
63-18	Light var.	0	0	0	2	2
63-19	Med. var.	1	7	0	9	17
63-20	Self red	0	0	1	2	3
			Ear 4[2]			
63-21	Light var.	1	1	0	3	5
63-22	Med. var.	0	0	0	2	2
63-23	Self red	0	0	1	0	1
			Ear 5[2]			
63-24	Light var.	1	0	0	1	2
63-25	Med. var.	0	4	0	5	9
63-26	Self red	0	0	5	5	10

[1]Parent genotypes = VV/WW × WW.
[2]Parent genotypes = VV/WR × WR.

often, at least, remote from the latter locus. The general conclusion to which the evidence leads is that a single germinal change takes place which affects two loci.

DISCUSSION

The leading fact disclosed by these experiments is that the mutable character variegated pericarp in maize may be differentiated regularly into two distinct phenotypes depending, not upon the variegated allele present, but upon a genetic unit separable from the *P* locus. Proof of the occurrence of such a unit is afforded by the data in tables 1 to 5 which show that the capacity to differentiate between light and medium variegated assorts more or less independently of *VV* and *WR* in the gametes formed by *VV/WR* heterozygotes.

It is proposed, for purposes of discussion, to call the newly recognized element Modulator, as descriptive of its regulatory effect on the variegated phenotype, and to apply the symbol *Mp* to it. The justification for considering Modulator as a genetic entity is that the phenotype from which the presence of the element is inferred is of a distinctive and recurring kind. If this criterion

alone were applied, the unit might be classified appropriately as a major modifying gene.

Modulator, however, is not a modifying gene of the usual sort; the second generation backcross data given in tables 1 to 4 rule out this possibility. One-half the families in this generation, from either the light variegated or the medium variegated seed ears, should have given one class of variegated offspring only on any of the standard assumptions which may be made concerning the distribution of a stable modifier among the parents. This is not what occurred. Among the 18 families from light variegated ears and the 30 families from medium variegated ears, there were none which did not include both kinds of offspring. Furthermore, the frequencies with which light and medium variegateds appear in these families are not compatible with the view that the differential between the two classes is a gene so incorporated in the hereditary complement that it is transmitted in a conventional manner.

Modulator may belong to the obscure category of germinal substances, termed heterochromatin, which has been associated through the work of MULLER (1930), SCHULTZ (1936), DEMEREC (1940), and others, with different variegated phenotypes in Drosophila. Variegated pericarp now is seen to conform in certain respects also to the genetic behavior of the mutable loci in the short arm of chromosome 9 in maize ingeniously analyzed by MCCLINTOCK (1950) and interpreted by her in terms of chromatin material accessory to the gene. The type of explanation of variegation which MCCLINTOCK has advanced may prove to be applicable to variegated pericarp, but our data are too limited to show how far the parallel extends.

The present results are understandable on the assumption that a common variegated gene is involved in the F_1 and backcross families, and that light variegated differs from medium variegated in the possession of Modulator, as a factor separate from the *P* locus. Derivation of the *VV* gene from a single heterozygous individual in the near ancestry of the foundation plant, S1937-10, and the uniformity of the two respective variegated phenotypes, light and medium variegated, are in accord with the view that one *VV* allele is present throughout.

The origin and germinal basis of Modulator are almost wholly obscure at present. No data are available concerning transmission of the element except in relation to *P*. The stability of the factor has not been studied, nor have the dosage effects been established. These are problems which can be clarified only by further experiments. There are certain regularities, however, in the rather complex body of data now at hand. It is important to inquire into the possible meaning of these facts if for no other purpose than to discover the directions in which a fuller understanding of Modulator may be sought.

The data from twin spots serve as an appropriate point of departure in constructing a working hypothesis. Adjacent self colored and light variegated areas on medium variegated ears heterozygous for colorless pericarp are so frequent that they must be regarded as the outcome of a single mutational

event. EMERSON (1917) demonstrated that the change from variegated to self color was due to a mutation at the *P* locus. The data presented in tables 1 to 5 show that light variegated, the co-twin to self color, differs from the parent phenotype, medium variegated, not in the *VV* allele present, but in the possession of Modulator at a locus separate from *P*. One may infer from this evidence that Modulator is a product of the transformation of *VV* to the stable self colored allele, *RR*.

It might be supposed that the variegated allele is an *RR* gene with which Modulator is associated at the *P* locus, the effect of *Mp* in this position being to suppress pigment formation. That is to say, *VV* may be equated to $\overline{RR\ Mp}$. Mutation of variegated to self color, on this hypothesis, would consist in the loss of Modulator from the *P* locus, thus restoring the pigment producing activity of the *RR* gene. Loss of Modulator at the *P* locus, when variegated mutates to self color, may be coincident, however, with acquisition of the same element elsewhere in the genome. It is assumed that Modulator at the new site plus the *VV* allele at the *P* locus gives rise to the light variegated phenotype. The medium variegated phenotype is the result of the action of the *VV* allele at the *P* locus, in the absence of *Mp* elsewhere in the complement. Modulator, except when present at the *P* locus, has no effect on the stable, self colored allele, *RR*. It is necessary to postulate also that the point at which Modulator becomes affixed, after removal from the *P* locus, varies.

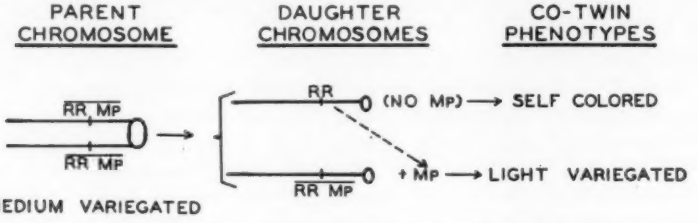
The three colored phenotypes occurring in segregating families thus may be represented as follows:

1) Self colored = *RR*
   Mp may be present in the genome, but not at the *P* locus and does not affect *RR* action.
2) Medium variegated = $\overline{RR\ Mp}$
   Modulator is present at the *P* locus only, and inhibits pigment formation.
3) Light variegated = $\overline{RR\ Mp} + Mp$
   This class differs from medium variegated in possessing a second Modulator unit which is not directly associated with the *P* locus, although it may lie in the same chromosome or in another chromosome.

If the new site which *Mp* occupies after removal from the *P* locus is at least 50 crossover units from *P* in chromosome 1, or is in another chromosome, then equal numbers of light and medium variegated offspring should result from mating heterozygous light variegateds with standard red cob colorless plants. On the other hand, if the new position to which *Mp* is transposed is near *P*, the resulting linkage will distort the ratio of light to medium variegated.

Twin spots can be assumed to originate in a mitotic division in which one of the two strands arising from the *VV* parent chromosome is *VV* (i.e., $\overline{RR\ Mp}$) and the other *RR*, at the *P* locus. That is to say, the Modulator component of *VV* is lost from one only of the two daughter chromosomes. Modulator is transposed in this process to a different site in the same or another chromo-

some. Also, the chromosome strand to which *Mp* is conveyed from the *P* locus must pass to the same daughter nucleus as the *VV* chromosomes, thus giving rise to the light variegated phenotype. The *RR* sector in a twin spot will thus lack Modulator. The following diagram illustrates this hypothesis:



The available twin spot data serve to test only one of the assumptions on which this model of the origin of Modulator is based. Is the supposition correct that the light variegated co-twin to self color is identical with the light variegated genotype to which the data in tables 1 to 4 apply?

The few data on hand on the genotype of the kernels in twin spots are presented in table 8. The plants bearing the five ears containing these twin spots were heterozygous for colorless pericarp, and were mated to colorless pericarp lines. The evidence bears on the composition of the *VV* and *RR* gametes only. Another generation is required to complete the separate test being made on the distribution of Modulator in the gametes bearing colorless pericarp. Twenty-three plants were obtained from the light variegated twin spot kernels, of which 15 proved to have colorless pericarp. Four of the plants with colored pericarp were light variegated, two were medium variegated, and two were self red. The numbers of plants are too few to be conclusive on the point, but the relatively high proportion of light variegated offspring is as expected if the twin spot kernels of this phenotype correspond to those on entire light variegated ears. Twenty-five plants with colored pericarp were obtained from the medium variegated control kernels on the twin spot ears. One of these plants had self colored pericarp, 23 were medium variegated, and only one was light variegated. The 21 self colored kernels from the twin spots gave nine self colored and 12 colorless offspring. It seems reasonably likely, therefore, that both the phenotypes appearing as co-twins are identical genetically with the same respective classes of whole ears. If the results from the tests now in progress on the constitution of the gametes bearing *WW* or *WR*, with reference to the differential between light and medium variegated, accord with those given above, it may be considered as proven that self color and light variegated arise concurrently from a single mutational event affecting two different loci.

It is apparent from the relevant data in tables 1 to 4, which are summarized in table 6, that among the offspring of medium variegated plants, self colored ears appear with about the same order of frequency as light variegateds. This

fact may be understood on the assumption that the light variegated and a corresponding number of the self colored ears are the result of complementary somatic mutations. The self colored class, however, is usually in excess. The difference may be due to Modulator frequently failing to become reestablished elsewhere in the chromosome complement after removal from the *P* locus. A mutational event of this kind would also explain the occurrence of self color mutant areas which are not twinned. An additional condition must be met, however, in the formation of a twin spot. The transposition of Modulator from the *P* locus to another site must be followed by passage of the recipient chromosome to the daughter nucleus which receives the *VV* strand, rather than the *RR* strand, of the dividing chromosome in which the mutation occurs. Presumably, Modulator is without effect on the self colored phenotype. Thus the appearance of light variegateds among the offspring of medium variegateds may be explained as the result of newly arisen mutations. Medium variegated does not contain Modulator as an element separate from the *VV* allele. It possesses the capacity to generate it, however, in conjunction with the transformation of *VV* to the self colored allele.

Consideration may next be given to the frequencies with which light and medium variegated and self colored ears occur among the offspring of light variegated plants. We have attempted below to account for the breeding behavior of the light variegateds in terms of segregation of Modulator considered as a relatively stable unit. It is clear that segregation is an operative factor in these families, but the extent to which the frequencies of lights and mediums are affected by somatic mutations of Modulator as well is not known. Mutations of light variegated to medium variegated occurring relatively early in ontogeny would be disclosed by the presence on the light variegated parent ears of large sectors of rather heavily variegated tissue. Such readily detectable mutant patches were not present on the ears in question. Late occurring somatic mutations of light to medium variegation, however, giving rise to mutant sectors involving a part of a kernel, often would not be recognizable because of the comparatively coarse nature of the variegated pericarp pattern. The effect of such late occurring mutations on the progeny of light variegated heterozygotes would be confounded with that of segregation at meiosis. Independent data on the stability of Modulator are needed to clarify this point. Meanwhile the possibility must be recognized that the factual basis for an interpretation of the breeding behavior of light variegateds may be incomplete in an essential respect.

The high proportions of light variegated progeny from heterozygous plants of this phenotype backcrossed to standard colorless pericarp (tables 1 to 4) are interpreted as the result mainly of segregation of Modulator, whose presence distinguishes this class of plants. If it were assumed that in light variegateds, *Mp* occupies a position 50 crossover units from *P* on chromosome 1, or were located on a non-homologous chromosome, and that no new mutations were occurring, then one-half the colored offspring of these test matings

should be light variegated. The frequencies observed in several families, particularly in the first and second generations of backcrosses to inbred line 8, approximate this value rather regularly. Thus it may be concluded that the site which *Mp* occupies after removal from the *P* locus frequently is remote from *P*.

Inspection of all the relevant data in tables 1 to 4, however, shows that the ratios from family to family in some backcross groups are far from homogeneous. Evidently *Mp* does not always assort independently of *VV*. Linkage between Modulator and *P* could account for the ratios obtained in certain families. On this hypothesis, the additional assumption must be made, however, that the site which Modulator occupies, after transposition from the *P* locus, varies widely. For example, in family 6-80 entered in table 2, all the variegated offspring are of the light class, indicating very close linkage between *VV* and *Mp*. The percentage of light variegated offspring in family 6-81 is 63.3 percent, corresponding to a rather loose linkage. The value drops to 12.7 in the third sister family, namely, 6-82. It is necessary to assume in the latter case that Modulator is coupled with *WR*, rather than *VV*.

The data in table 5, showing the distribution of Modulator in relation to *WR* among the offspring of light variegated *VV/WR* plants mated with *VV/WW* individuals are explainable in the same terms. The proportions of light variegated plants, although clearly set off, for the most part, from the corresponding group of data from the medium variegated sibs, are notably variable, even between certain sister families. In spite of the very close relationship between N124, N125, and N127, for example, these families contained 56.7 percent, 28.6 percent, and 18.2 percent, respectively, of light variegated plants. The average scores for variegation grade of the progeny ears show clearly that the same two classes of offspring, light and medium variegated, are present throughout. That is to say, the three families differ only in the proportions in which these two phenotypes occur. On the supposition that linkage is the principal factor determining the distributions it is necessary to postulate that Modulator occupied a different site in each case.

It is quite clear from these several considerations that Modulator cannot be explained in conventional genetic terms either in respect to origin or mechanism of inheritance. There are other facts, not presented in this report, which must be brought into relationship with each other and with the present evidence before a general interpretation of the phenomenon can be undertaken profitably. For example, ANDERSON (1924) has shown that variegated mutates to colorless pericarp as well as to self color, although much less often. These white mutations have not been studied, and the mating plan used in the present investigation would not disclose them. Furthermore, it is evident from unpublished data which we have obtained that more than one kind of *P* allele occurs conditioning the so-called calico type of variegation which characterizes the present stocks. The basis for any general conclusions obviously will remain incomplete until the effects on the variegated phenotype of major variations both at the *P* locus and elsewhere in the genome can be integrated. A further

well established fact which remains unexplained is the higher rate of mutation of *VV* to *RR* in *VV/WR* heterozygotes than in *VV/VV* homozygotes. These are some of the reasons why discussion of the problem is necessarily speculative at this stage. A certain orderliness in the experimental results appears, nevertheless, when the difference between light and medium variegated pericarp is looked at from the point of view outlined above. No more is claimed for the hypothesis offered, however, than that it serves currently as a basis for continuing the analysis.

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SUMMARY

1. Three distinct pericarp phenotypes, light and medium variegated, both of which are unstable, and self colored, which is stable, were obtained in each F_1 family resulting from the application of a single collection of pollen from a particular homozygous variegated (*VV/VV*) maize plant to a series of different inbred strains having either colorless pericarp, red cob (*WR/WR*) or colorless pericarp, white cob (*WW/WW*). The average proportions in which the three phenotypes appeared were 10.7 percent, 72.4 percent, and 16.8 percent, respectively.

2. Breeding tests were made designed to disclose the inheritance conditioning variegated pericarp transmitted through (1) the *VV* gametes and (2) the *WR* gametes, formed by the light variegated and medium variegated F_1 sib plants. The data on the *VV* gametes were obtained by backcrossing through two successive generations to the appropriate inbred line. Composition of the *WR* gametes was inferred from the character of the red cobbed offspring arising from the F_1 *VV/WR* plants after mating with unrelated *VV/WW* individuals.

3. Most of the offspring within the relevant classes, obtained from the medium variegated plants, were medium variegated, following both types of test matings. That is to say, the potentiality for determining the medium variegated phenotype was transmitted through a high proportion of both the *VV* and *WR* gametes. A few light variegated plants also appeared regularly in these families, together with a somewhat higher proportion of self colored individuals.

4. In contrast, the light variegated plants gave relatively high proportions of light variegated offspring in both kinds of test matings. The frequency of medium variegated plants was correspondingly reduced; and the proportion of self colored individuals also was lowered.

5. Counts made on medium variegated ears showed that a patch of mutant self colored tissue was frequently twinned with a light variegated area. The limited breeding data available indicate that light variegated and self colored

co-twins correspond to these respective classes of kernels as they occur on entire ears. If this finding is confirmed it means that self color and light variegated arise concurrently from a single mutational event affecting two different loci.

6. As a working hypothesis, it may be assumed that the unstable variegated allele, *VV*, is a modified form of the stable *RR* gene, the difference between them being that *VV* embodies a discrete element, termed Modulator, *Mp*, which inhibits pigment formation. Mutation of *VV* to *RR* consists in the loss of *Mp* from the *P* locus.

7. Following removal from the *P* locus, *Mp* may become attached at one or another position elsewhere in the chromosome complement. Modulator thus situated, plus *VV*, gives the light variegated phenotype. *VV* alone conditions the medium variegated phenotype.

8. Modulator cannot be explained in conventional genetic terms either with respect to origin or mechanism of transmission. At this stage an interpretation on any other basis is necessarily speculative.

9. The relatively few light variegated and self colored offspring of medium variegated plants are interpreted as carrying newly arisen mutations. The character of the families from light variegated plants is conditioned both by such mutations and the segregation of *Mp* as a unit separate from the *P* locus.

10. The varying proportions of light and medium variegated offspring of light variegated plants are interpreted in terms of linkage or independence of Modulator relative to the *P* locus.

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THE LOWERING OF THE MUTAGENIC EFFECTIVENESS OF ULTRAVIOLET BY PHOTOREACTIVATING LIGHT IN DROSOPHILA

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ALTHOUGH there is now (since we started our present work) evidence that long ultraviolet and visible light lower the mutagenic effectiveness of ultraviolet in certain microorganisms (DULBECCO 1950; GOODGAL 1950), we have thought it desirable to carry through our investigation of this effect in Drosophila, both because considerable differences in mutagenesis, of other kinds, have been found between widely different organisms and because the deleterious effects of mutations might be greater when induced in haploid material (as in microorganisms) than in diploid and these effects might cause complications in conjunction with the physiologically deleterious effect of ultraviolet.[1]

MATERIALS AND METHODS

It will be recalled that the polar cap appears at one pole of the developing Drosophila egg shortly after fertilization and that it contains the cells of the early germ track. About 55 cells are present in the fully developed polar cap of a male egg, but only some 20 to 24 of these become incorporated in the testes when these are formed (SONNENBLICK 1941, 1951). The polar cap is separated from the outside by only the thin transparent vitelline membrane and by the chorion (shell) of the egg. The polar cap cells are therefore almost directly accessible to ultraviolet that strikes the overlying surface, particularly when the shell of the egg is removed. It is impractical to treat the adults with ultraviolet, because only a small fraction of the ultraviolet that strikes the surface of the adult fly can penetrate the thickness of tissue that separates the gonads from the outside surface.

The polar caps of two lots of Drosophila eggs were treated with ultraviolet from a 15-watt germicidal lamp. One lot was given no further treatment. The other lot was posttreated with photoreactivating light, the source of which was a 100-watt General Electric CH–4 spotlight lamp, a large percent of the output of which was radiation ranging in wave length from 3000 to 4200 Å. The posttreatment usually lasted for 30 minutes (but only for three minutes in one case) and it followed immediately after the mutagenic ultraviolet treatment. In one series of experiments (Series 1), the shells were not removed from the

[1] We were aware that, while we were doing the present work at The Rice Institute, a parallel investigation was being carried out at Indiana University (MEYER 1951), but both groups were of the opinion that in this case, owing to the difficulties involved, it was desirable to have two separately obtained sets of results. By mutual agreement, these are being published in parallel papers.

eggs previous to treatment, neither was the light from the germicidal lamp filtered. In two other series, the shells of the eggs were removed and the light was passed through a filter (consisting of an aqueous solution of $CoSO_4$ and $NiSO_4$), so as to render it mostly monochromatic ultraviolet of wave length 2537 Å. This was done in order to eliminate all photoreactivating light from the germicidal lamp, but as it turned out, this precaution was unnecessary, as shown by the results (to be considered later) of Series 1 experiments in which the light was not filtered.

Second chromosome lethals were used as a measure of the mutation rate. These were detected by means of MULLER's "sifter" technique (MULLER 1951), which is being fully described in another article now in preparation, but since the technique is new and complicated, it will be reviewed here. In the form used here, the technique is briefly as follows. The treated polar caps are those of eggs of genotype *cn bw sp/cn bw* (*cn* = cinnabar, *bw* = brown, *sp* = speck). When the eggs reach maturity the males (termed P_1) are selected and mated to *Cy cn² sp²/mr bs* females (*Cy* = Curly, *mr* = morula, *bs* = blistered). The Curly offspring are of two classes (1) *cn bw sp/Cy cn² sp²* (phenotypically Curly cinnabar speck) and (2) *cn bw/Cy cn² sp²* (Curly cinnabar non-speck). About 20 F_1 brothers of each of these two classes, or some other convenient number, derived from each P_1 male are selected as P_2 and individually bred to females of a complicated stock designated by MULLER as "sifter."

The sifter stock is heterozygous for two Oenothera-like complexes which involve rearrangements of the second and third chromosomes. One of these complexes contains *Cy*, and we shall designate this as the *Cy* sifter complex. The other complex contains P^-, the Pale deficiency. The sifter stock is so constituted that in outcrosses, the only viable offspring are those that receive the *Cy* sifter complex. (Those that receive the P^- complex are inviable because of the Pale deficiency. The stock itself is viable because the *Cy* sifter complex contains P^t, the Pale insertion, which compensates for the Pale deletion. Crossovers between the two complexes are suppressed by inversions and chromosomal recombinations are made inviable by the unbalance they cause, sifter stock being a translocation heterozygote). Accordingly, when the P_2 males are bred to sifter females, the only viable offspring are those that receive the Curly chromosome from their sifter parent. Therefore they cannot also receive the Curly chromosome of the non-sifter parent (the P_2 male), since homozygous *Cy* is inviable. Thus they all receive his treated chromosome. If we indicate either treated chromosome by the symbol, *cn bw* (*sp*), then the P_2–F_2 cultures are as follows:

P_2 *cn bw* (*sp*)/*Cy cn² sp²* ♂ × sifter ♀ (with *Cy* sifter complex)
F_2 *cn bw* (*sp*)/*Cy* sifter complex ♂ and ♀.

It thus comes about that all the F_2 are of the desired constitution for further breeding; i.e., all are heterozygous for a second chromosome of treated origin as well as for a marker and crossover suppressors in the nontreated chromosome. (We shall designate the latter as the sifter *Cy* chromosome.)

A few of the F_2 are now taken from each culture and inbred (as P_3) without the selection of virgins being necessary (since all the F_2 are of the right class). The flies from different cultures are kept separate, so that there are about 20 P_3–F_3 cultures of *cn bw sp/Cy* sifter and 20 of *cn bw/Cy* sifter ♂ and ♀ for each treated polar cap (corresponding to the 20 P_2–F_2 cultures from which they were individually derived). If the P_3 do not contain a lethal in their treated chromosome (*cn bw sp* or *cn bw*), they produce offspring homozygous for this chromosome in addition to *Curly* flies heterozygous for it, thus:

P_3 *cn bw (sp)/Cy* sifter ♂ and ♀
F_3 1 *cn bw (sp)/cn bw (sp)* : 2 *cn bw (sp)/Cy* sifter.

The *cn bw* combination when homozygous (and when not modified as described below) produces white eyes, and the nonlethal cultures can therefore be scored at a glance simply by observing a few white-eyed flies through the glass container. About two-thirds of the offspring homozygous for the *cn bw* combination have cinnabar eyes instead of white, because the P_2 sifter parent carried the Pale insertion in one of its third chromosomes and this insertion contains the normal allele of *bw* (and of *sp*), thus allowing only *cn* to express itself in those F_3 that have received the insertion. The presence of *cn* flies (if straight-winged) would therefore also indicate that the culture was nonlethal. All flies that had straight wings would be homozygous for *cn bw (sp)*, so that the presence of any straight-winged flies in the culture would identify it as nonlethal. But the white eye is the most convenient trait for identifying the nonlethal cultures, since it is very conspicuous. The lethal cultures on the other hand would lack the whites and contain only the heterozygotes, *cn bw (sp)/Cy* sifter. These would have dark cinnabar eyes (cn^2 being contained in the *Cy* sifter complex) and Curly wings. A summary of the above crosses is as follows:

P_1 *cn bw/cn bw sp* ♂ × *Cy* cn^2 sp^2/*mr bs* ♀
(♂ irradiated in polar cap stage)
P_2 20 brother F_1 *cn bw/Cy* cn^2 sp^2 ♂♂ and
20 brother F_1 *cn bw sp/Cy* cn^2 sp^2 ♂♂ from each P_1 ♂
bred individually × sifter (with *Cy* sifter complex) ♀♀
P_3 *cn bw/Cy* sifter complex ♂ and ♀; *cn bw sp/Cy* sifter complex ♂ and ♀
(20 cultures) (20 cultures)
F_3 Look for *cn bw/cn bw* or *cn bw sp/cn bw sp*

Any lethal which arises in a polar cap cell is multiplied by mitotic cell division to the same extent that the cell is. Thus if a polar cap contained 10 cells, and a lethal arose in one of these, say in the *sp* chromosome of the cell in question, and further if each cell of the polar cap multiplied equally, then on the average one-tenth of the sperm cells with a *sp* chromosome (i.e., one-tenth of the *sp* genome) would contain the lethal as a result of reduplication of the one original lethal. In other words, there would be a cluster of lethals equal to one-tenth of the sperm cells with the *sp* genome. Therefore if, say, 20 of the sperm cells with the *sp* genome were tested for a lethal, then a cluster of two

(1/10 of 20) would on the average contain the lethal, on the above assumptions. (As a matter of fact, the number of germ cells per polar cap might not be 10 at the time of irradiation; and the division rate of all cells is not the same.)

In the sifter technique as here employed, it will be recalled that about 20 chromosomes of each of the second chromosome pair (*cn bw sp* and *cn bw*) are tested from each treated polar cap, and if a lethal has been induced in, say, just one cell of a polar cap and in one of the second chromosomes of that cell, then a certain fraction of the 20 P_3–F_3 cultures containing that kind of second chromosome will contain the lethal, and the rest will not. The size of the fraction (cluster) with the lethal will depend on the number of germ cells in the polar cap at the time the lethal arose, and on the extent to which the mutant cell multiplies, relative to the others, as indicated above.

When a polar cap contains just one mutant cell, it is a mosaic of mutant and normal cells, and this is reflected by the fractional transmission of the mutant chromosome to the offspring. If, on the other hand, all the cells of a polar cap contained a lethai, and at the same locus, in one of the second chromosomes, then the embryo would most likely have been heterozygous for that lethal at the time it was a fertilized egg, and we should then not consider that lethal as having been induced in the polar cap, but as preexisting (at the time of fertilization). Such lethals are not included in the totals.

Since the P_2 males derived from a given treated polar cap (of a P_1 male) are brothers, we can refer to the P_2–F_2 cultures in which they are the fathers as "brother" cultures, those with the *cn bw sp* chromosome being referred to as one series of brother cultures (the "speck" series) and those with the *cn bw* chromosome as another series (the "non-speck" series). The P_3–F_3 cultures (derived from a given treated polar cap) would be correspondingly designated. In this terminology, then, a P_3–F_3 series which is partly lethal represents a new lethal; one which is all-lethal represents a preexisting lethal.

Two or more lethals might be induced in the same polar cap (lethals of "independent origin"). When flies with two such lethals are crossed, offspring homozygous for the *cn bw* (*sp*) chromosome (i.e., whites) might survive, since the lethals would most likely be at different loci, and each would be prevented from expressing itself by its normal allele in the other chromosome. By contrast, two flies with lethals derived from the same original mutation could produce no offspring homozygous for *cn bw* (*sp*), since the lethals in this case would be alleles. Crosses were always made to test the allelism of the lethals in a given P_3–F_3 series (by crossing flies from one culture to those of all other cultures of the series).

When a mutation is induced in a polar cap cell, the nonmutant cells are multiplied on the average to the same extent (per cell) as the mutant. Therefore, the ratio of mutant to nonmutant chromosomes is the same in the adult as in the polar cap. Thus a cluster of mutant sperm cells equal, for example, to ten percent of the total sperm cells with a given second chromosome (*sp* or *non-sp*) would have resulted (on the average) from one mutant chromosome

in a total of ten germ-track cells in the polar cap and would therefore represent a ten percent mutation rate for that chromosome. Hence even though the cluster was derived from only one original mutation, the cluster must nevertheless be regarded as representing a ten percent mutation rate. This in turn means that each mutation in the cluster is scored as though it were independently induced in the sperm cells themselves, so that if for example a total of 100 sperm cells were scored (through the appropriate genetic tests) and ten found to be mutant, the mutation rate would be set down as ten percent, although all ten mutant sperm cells might belong to a single cluster derived from only one mutational occurrence.

The cells of the fully developed polar cap do not proliferate germ cells equally for very long. After they wander to the site of the future testis and become gonial cells, they undergo only very few if any equal germinal divisions. Thereafter one or a few of the cells (stem cells) in each testis do most of the germinal multiplying. Hence if one of these particular cells happened to contain an induced mutation, the estimate of the mutation rate, based on the size of the resulting cluster, would be much higher than if one of the other polar cap cells had happened to contain the mutation. This unequal division of the gonial cells therefore introduces a sampling error in the estimate of the mutation rate, for if the number of polar caps treated is small, the rate in two samples of equal size might vary considerably depending on the number of stem cells hit, even though the same percent of polar cap cells had been hit in the two samples.

It was not usually possible to derive the desired 20 P_3–F_3 cultures with each kind of treated chromosomes (*cn bw* and *cn bw sp*) from each P_1 male, partly because of sterility of the treated males and partly because of accidents. When a male happened to be unusually fertile, more than 20 cultures of each kind were sometimes derived from him in order to augment the data.

The method of estimating the sampling error herein used has been developed by MULLER. To get the standard error by this method we square the number of lethals in each cluster (an isolated lethal being considered a cluster of one), get the sum of these squares, multiply this sum by the percent of nonlethals, take the square root of this product, and divide this by the total number of tested chromosomes derived from all the treated polar caps.

In order to estimate the sampling error, it is necessary to know the number of tested chromosomes and lethals derived from each treated polar cap (of the P_1 male); but the separate presentation of the data derived from each treated male would require an excessively bulky table. In preparing table 1, therefore, the data from all males receiving a given treatment were lumped together.

RESULTS

Table 1 Series 1 gives the results of experiments in which neither the shells of the eggs were removed before treatment nor the ultraviolet light (from the germicidal lamp) filtered. It will be seen that the rate in the lot of eggs treated with mutagenic ultraviolet alone (1D) is $7.3 \pm 2.3\%$; in the posttreated lot

(1L), $1.0 \pm 1.0\%$. The latter is very close to the control rate of $.8 \pm .3\%$. Thus the photoreactivating light has largely abolished the mutagenic effectiveness of the ultraviolet.

Table 1 Series 2 gives the results of experiments in which the shells were removed from the eggs prior to treatment and in which also the light from the germicidal lamp was rendered almost monochromatic, of wave length 2537 Å, by being passed through a filter. In this series of experiments, the eggs received a very low dose of mutagenic ultraviolet (about 835 μw-sec/cm^2), sufficient to give a gross mutation rate of only $2.7 \pm .7\%$, which however is still

TABLE 1

Effect of photoreactivating light of ultraviolet mutagenesis in Drosophila.

D = Dark (placed in dark after mutagenic ultraviolet treatment)
L = Light (treated with long ultraviolet and visible light for 30 minutes after mutagenic ultraviolet treatment; L1, for only 3 minutes)

Series*	% hatched eggs ♂♂**	Approx. % P_1 ♂♂ sterile***	No. polar caps tested	No. chromosomes tested	No. new lethals	No. independent lethals	Mutation rate (%)	Induced rate (%)	Diff. (%)
1D	9.6	31.2	38	1,242	100	24	8.1 ± 2.3	7.3 ± 2.3	6.3 ± 2.5
1L	17.8	20.0	32	924	17	8	1.8 ± 0.9	1.0 ± 1.0	
Controls	38.0	2.6	36	1,432	11	10	.8 ± 0.3		
2D	34.4	8.1	37	1,252	34	20	2.7 ± 0.7	1.9 ± 0.8	
2L	32.0	13.2	35	1,050	17	13	1.6 ± 0.5	.8 ± 0.6	
2L1	22.2	0.0	10	408	5	5	1.2 ± 0.5	.4 ± 0.4	
Controls	41.6	4.8	20	648	5	5	.8 ± 0.3		
3D	31.7	5.3	17	528	52	28	10.0 ± 2.0	9.5 ± 2.0	5.4 ± 2.7
3L	18.8	6.3	12	326	15	9	4.6 ± 1.7	4.1 ± 1.8	
Controls	40.5	6.7	11	220	1	1	.5 ± 0.4		

*Series 1 (1D and 1L). Ultraviolet not filtered. Shells not removed from eggs. Distance of eggs from ultraviolet lamp, 87¼ cm. Duration of ultraviolet treatment, 19 minutes; of posttreatment, 30 minutes. Dose of ultraviolet, 37,500 μw-sec/cm^2 (only 10% of which penetrates shell).
Series 2 (2D, 2L, 2L1). Filtered ultraviolet. Eggs without shells. Distance of eggs from ultraviolet lamp, 6 cm. (filter greatly lowers intensity of ultraviolet). Duration of ultraviolet treatment, 2 minutes; of posttreatment, 30 minutes (L) and 3 minutes (L 1). Dose of ultraviolet, 836 μw-sec/cm^2.
Series 3 (3D, 3L). Ultraviolet filtered, but with deteriorating filter (dose of ultraviolet therefore uncertain). Treatment otherwise as for Series 2, with 30 minutes posttreatment.
**Percent of hatched eggs that developed into males.
***Including possibly a few males that were fertile but that failed to breed.

significantly above the control rate ($.8 \pm .3\%$), the difference between the treated and control rates being $1.9 \pm .8\%$ ($t = 2.5$). By contrast, the gross rate for the posttreated lot ($1.6 \pm 0.5\%$) is not significantly different from that for the controls.

One batch of eggs intended for ultraviolet treatment only, in Series 2 experiments, was inadvertently placed in the photoreactivating light and given a 3-minute posttreatment (2L 1) before the error was discovered. The experiment was nevertheless continued with these eggs, and it was found that the 3-minute posttreatment was sufficient to cause a significant lowering of the

mutation rate, as judged by the fact that the eggs in question gave an induced mutation rate of $.4 \pm .4\%$, as compared with a rate of $1.9 \pm .8\%$ for the previously mentioned ultraviolet only treatment (2D) in Series 2 experiments. (The usual posttreatment lasted for 30 minutes, it will be recalled.)

In Series 3 experiments (done before Series 2), it was discovered only late in the course of the experiments that the filter was deteriorating, and that after being used a few times it allowed much more ultraviolet to pass through it than it did at the start. (The filter was renewed before each treatment in Series 2 experiments). However, in each experiment of Series 3 (with the deteriorating filter), a comparable number of chromosomes were usually scored for the non posttreated (3D) and the posttreated lot of eggs (3L), and since the two lots would have received the same amount of mutagenic ultraviolet in each experiment, the average amount received by the two for the entire Series 3 experiments would have been about the same. Regardless of what this average dose may have been, there is a significantly lower induced rate in the posttreated ($4.1 \pm 1.8\%$) than in the ultraviolet-only treated eggs ($9.5 \pm 2.0\%$). However, the rate for the posttreated in this series ($4.1 \pm 1.8\%$) is significantly above the control rate ($.5 \pm .4\%$).

TABLE 2

Number of independent lethals per tested polar cap.

Series	D (u.v. only)				L (u.v. plus photoreactivating light)				Controls		
	No. polar caps	Independent lethals			No. polar caps	Independent lethals			No. polar caps	Ind. lethals	
		No.	No. per polar cap	No. induced		No.	No. per polar cap	No. induced		No.	No. per polar cap
1	38	24	.63	.35	32	8	.25	....	36	10	.28
2	37	20	.54	.29	35	13	.35	.10	20	5	.25
3	17	28	1.65	1.56	12	9	.75	.66	11	1	.09

The number of independent lethals induced per treated polar cap (counting each mutant cluster as one), is significantly less for the posttreated than for the non posttreated (ultraviolet only) polar caps in all three series of experiments (table 2). This method of treating the data therefore confirms the conclusion based on estimating the mutation rates from the percent of lethals per tested chromosome shown in table 1.

TABLE 3

Average size of lethal clusters, expressed as the percent of tested chromosomes of each kind (cn bw sp and cn bw) that are lethal, per lethal-yielding polar cap.

Series	D	L	Controls
1	21	18	7
2	11.5	8	5
3	17	9	9

There is somewhat of a tendency for the average size of a lethal cluster to be smaller in the posttreated than in the non posttreated polar caps, though this difference may not be significant (table 3). (The control lethals have a small average cluster size because most of these would not have arisen in the polar cap stage but later, and would therefore have been reduplicated less than lethals induced in the polar cap.) The smaller average cluster size for the posttreated agrees with the fact that the number of cells surviving per polar cap would be expected to be greater in the posttreated (because of the physiological photoreactivation) than in the non posttreated polar caps, and this would tend to make the cluster size (relative to the total surviving cells) smaller for the posttreated. However, the increase in number of surviving cells per polar cap would make the number of independent lethals per polar cap greater in the posttreated, if the mutation rate per cell was the same as in the non posttreated, so that the smaller number actually found for posttreated (mentioned in the paragraph above) now requires all the more significance.

DISCUSSION

The evidence clearly shows that photoreactivating light lowers the mutagenic effectiveness of ultraviolet in Drosophila, and that it may lower it to the extent of almost completely abolishing it. Photoreactivating light thus has an even greater inhibiting action on ultraviolet mutagenesis than on the physiologically toxic effect of ultraviolet in bacteria.

The mechanism whereby photoreactivating light lowers the mutagenic effectiveness of ultraviolet still remains to be explained. One *a priori* possibility is that it reverses the mutation process itself, either before the process is completed or after. The other is that the photoreactivating light has no effect whatever on mutation proper, but that it simply prevents this process from occurring, possibly by neutralizing some mutagenic product of ultraviolet before the product in question has time to become effective. The second of these possibilities seems the more probable, since if photoreactivating light could reverse a mutation, it would itself in effect be mutagenic, and this we know is not ordinarily the case.

SUMMARY

The polar cap of two lots of Drosophila eggs were treated with approximately the same dose of mutagenic ultraviolet (mostly of 2537 Å wave length), one lot then receiving no further treatment, the other receiving a posttreatment (usually for 30 minutes) with photoreactivating light (mostly in the range 3000–4200 Å). The lethal mutation rate induced in the second chromosome (as scored by MULLER's sifter technique), was lower in the posttreated lot than in the non posttreated, in each of three separate series of experiments. In two of the three series (Series 1 and 3) involving medium doses of mutagenic ultraviolet, the rate for the posttreated lot was significantly below that for the non posttreated; and in one series (Series 2) involving a very light dose of mutagenic ultraviolet, though the difference between the two rates

(for non posttreated and posttreated) was not highly significant (because of the low rates involved), yet the rate for the non posttreated lot was significantly above the control rate, but that for the posttreated was not. In Series 1 the rates were as follows: non posttreated 7.3 ± 2.3% (1,242 tested chromosomes); posttreated 1.0 ± 1.0% (924 chromosomes); controls .8 ± .3% (1,432 chromosomes). In Series 2 experiments the rates were: non posttreated 1.9 ± .8% (1,252 chromosomes); posttreated .8 ± .6% (1,050 chromosomes); controls .8 ± .3% (648 chromosomes). For Series 3 the figures were: non posttreated 9.5 ± 2.0% (528 chromosomes); posttreated 4.1 ± 1.8% (326 chromosomes); controls .5 ± .4% (220 chromosomes). Thus in two of the three series the induced rates for the posttreated chromosomes is very close to the control rates, even though in Series 1 the induced rate without posttreatment was fairly high (7.3 ± 2.3%). Photoreactivating light therefore has at these doses an even greater inhibiting action on the mutagenic effectiveness of ultraviolet than on the physiologically toxic effect reported for bacteria.

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THE NON-EFFECT OF INTENSITY ON THE MUTAGENESIS OF ULTRAVIOLET LIGHT WITHIN A NINETEEN-FOLD RANGE IN DROSOPHILA

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THE mutation rate induced in Drosophila by ultraviolet light does not increase proportionately with increase in dose of ultraviolet light, especially at the higher doses employed (ALTENBURG *et al.* 1950). The reason for this is not obvious. It was thought that some light might be shed on the relationship between the dose of ultraviolet and the induced mutation rate by determining whether or not a given dose of ultraviolet produces the same mutation rate, regardless of its intensity. The possibility that intensity might influence the mutation rate was suggested by some early work which indicated that low intensities were less damaging to the adult Drosophila than were high per given dose of ultraviolet light (ALTENBURG 1930). Low intensities were also found to be less damaging to the developing embryo and to the cells of the early germ track (MEYER and MULLER, personal communication). Thus, since the physiological damage done by ultraviolet is partly dependent on its intensity, the genetic damage might conceivably also be.

MATERIALS AND METHODS

The germ track of Drosophila was treated with ultraviolet at the polar cap stage (see ALTENBURG and ALTENBURG 1952). The chorions of the eggs were not removed. The polar caps were illuminated from "above"; that is, the rays of ultraviolet were parallel to the polar axis of the egg (or at right angles to the equatorial plane). Some of the eggs were taken as samples and examined under the microscope immediately before treatment (and sometimes after also) to see that they were in polar cap during treatment. The source of the ultraviolet light was a 15-watt germicidal lamp.

Several experiments were run, and in each one the polar caps of two lots of eggs were given the same dose of ultraviolet but of different intensity. The lot which received the lower intensity got a correspondingly longer treatment so as to make the total dose the same for the two lots. The intensity was varied by placing the two lots at different distances from the source, those farther away of course receiving the lower intensity.

Two separate series of experiments were run (Series I and II). The two series were done at different times of the year and Series II, the smaller of the two series, was actually done before Series I and was repeated, with slight modification, because the results of this series did not seem quite conclusive. In each series the set-up was essentially as described above, but the relative intensities of ultraviolet in the two series were somewhat different. In Series I

one lot of eggs was placed at 18 cm from the source, the other at 87¼ cm, and the resulting relative intensities were as 19 : 1 (for near and far treatments, respectively). The "near" eggs were treated for one minute; the "far" for 19 minutes. The dose of ultraviolet received by each lot of eggs as measured by a General Electric ultraviolet meter was approximately 38,000 μw-sec/cm^2. In Series II experiments one lot of eggs was placed at 25 cm from the lamp, the other at 80 cm. The relative intensities at the two distances were as 10 : 1 (for near and far, respectively). The duration of the treatment was 1.5 minutes for the near eggs and approximately 15 minutes for the far, the two lots of eggs therefore receiving the same total dose of ultraviolet (about 38,000 μw-sec/cm^2).

After the treated (and control) eggs developed into adults, the males were selected and bred in order to determine the mutation rate. Lethals in the second chromosome were used as a measure of the mutation rate. These were detected by MULLER's "sifter" technique (ALTENBURG and ALTENBURG 1952). The problems of interpretation of mutation rates from irradiated polar cap cells have been discussed by ALTENBURG and ALTENBURG (1952).

The treatment of the eggs at the polar cap stage is rather laborious and must be completed within the very limited time that the eggs are in this stage. Moreover, many of the eggs that are treated are lost because they are of the wrong sex, or are sterile, or are damaged in handling. For this reason only a relatively few eggs might have been successfully treated and bred in a given experiment, and though an attempt was always made to get about the same number of treated males for the "far" and "near" treatments and for the controls, yet it sometimes happened that no males were got from one kind of

TABLE 1

The mutagenic effectiveness of high intensity ultraviolet ("near") as compared with low ("far").

Distance	No. ♂♂	Number of chromosomes	Mutation rate	Induced mutation rate	Far-near difference	Number of independent lethals per genome
			Series I			
Near	36	1,239(97)	7.8 ± 2.0%	6.9 ± 2.0%	.9 ± 2.4%	.50 ± .06%
Far	38	1,715(118)	6.9 ± 1.7	6.0 ± 1.7		.45 ± .06
Controls	55	2,179(19)	.9 ± 0.2	....		.15 ± .03
			Series II			
Near	20	641(31)	4.8 ± 2.2	3.4 ± 2.0	2.7 ± 3.4	.23 ± .07
Far	18	533(40)	7.5 ± 2.7	6.1 ± 2.7		.36 ± .08
Controls	28	836(12)	1.4 ± 0.7	....		.11 ± .01
			Series I and Series II			
Near	56	1,880(128)	6.8 ± 1.5	5.8 ± 1.5	.2 ± 2.1	.40 ± .05
Far	56	2,248(158)	7.0 ± 1.4	6.0 ± 1.4		.41 ± .05
Controls	83	3,015(31)	1.0 ± 0.2	....		.13 ± .03

treatment and successfully bred but were from the other, as when for example all those from the " near " treatment were lost but not all from the " far " or from the controls. However, the experiments (of a given series) were usually done on consecutive days and with the same stocks, so that on the whole the " near " and " far " treatments were done under similar conditions.

RESULTS

The detailed data are too voluminous for inclusion here, but are summarized in table 1. The standard errors have been computed by the method of MULLER (see ALTENBURG and ALTENBURG 1952). It will be seen that in the case of the Series I experiments, the difference between the induced rates for the near and far treatments ($0.9 \pm 2.4\%$) is not significant; neither is the difference in the case of the Series II experiments ($2.7 \pm 3.4\%$). When both series are combined, the difference between the two rates (0.2%) is still less significant than it is in the case of either series alone. When the rates are calculated on the basis of the number of independent lethals per polar cap or per genome, it is again seen that there is no significant difference in rates.

DISCUSSION

If the mutagenic action of ultraviolet were some sort of multiple hit effect, and moreover if the effect of each hit were of short duration (shorter than the duration of the treatment), then one might expect higher intensities of ultraviolet to have a greater mutagenic effect than lower intensities, since the higher intensities favor coincidences of two or more hits, per given dose, within a limited interval of time. However, if the effect of a hit were of long duration, then intensity might be without effect, even on a multiple-hit theory. The present results therefore indicate that the multiple hit theory either does not apply to ultraviolet mutagenesis, or that the effectiveness of each hit lasts longer than the interval over which the lower intensity was employed. However, results of other experiments indicate that within the lower dosage range employed in these experiments, the mutation rate is an exponential function of the dose. This would indicate that fundamentally the mutagenic action of ultraviolet is a multiple hit effect. Therefore, the second alternative just given (postulating a long-lasting effect of each hit) would more likely account for the non-effect of intensity on the mutation rate.

SUMMARY

No significant difference was found in the mutagenic effectiveness of ultraviolet within a nineteen-fold variation of intensity (when the total dose was kept the same), as measured by the lethal mutation rate induced in the second chromosome of Drosophila by treatment of the germ track at the polar cap stage in the development of the egg. The lethals were detected by MULLER'S sifter technique. In one series of experiments the higher intensity was ten times that of the lower and in a second series the higher was 19 times that of the

lower. In all experiments combined, the induced mutation rate at the lower intensity was $6.0 \pm 1.4\%$ (based on 2,248 tested chromosomes), at the higher intensity $5.8 \pm 1.5\%$ (1,880 chromosomes), control rate $1.0 \pm .2\%$ (3,015 chromosomes). Thus the difference (0.2%) between the effectiveness of the low and high intensities is not significant.

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EVIDENCE INDICATING THAT THE MUTATION RATE INDUCED IN DROSOPHILA BY LOW DOSES OF ULTRAVIOLET LIGHT IS AN EXPONENTIAL FUNCTION OF THE DOSE

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IN Drosophila (as in fungi) the frequency of mutations induced by ultraviolet light does not increase proportionately with increase in the dose (HOLLAENDER *et al.* 1945; ALTENBURG *et al.* 1950). However, there was some indication from the work on Drosophila that at the lower doses employed, the induced rate tended to be proportional to the dose (ALTENBURG *et al.* in manuscript). Further experiments were therefore undertaken to determine the shape of the dosage–rate curve at the lower doses.

METHODS

The germ track of Drosophila was treated with ultraviolet light at the polar cap stage in the development of the fertilized egg. The chorions of the eggs were removed before treatment and the polar cap ends of the eggs were illuminated from "above" (ALTENBURG and ALTENBURG 1952). The eggs were examined under the microscope just before and after treatment to see that they were in polar cap. During treatments the eggs were kept in a cold chamber to slow down their development and prevent them from getting past the polar cap stage before the completion of the treatment.

A number of experiments were run, and in each experiment four lots of eggs were treated at 360 cm from the lamp. One lot was treated for 8 minutes, the second lot for 16 minutes, the third 24 minutes, and the fourth 32 minutes. Thus the relative doses were as 1, 2, 3, and 4. The dose of ultraviolet as measured by a General Electric meter was 800 μw-sec/cm^2 for the lot receiving the 8 minute treatment, the remaining lots receiving 2, 3, and 4 times this dose, respectively. Previous experiments had shown that a dose about equal to dose 1 here employed was close to the lowest which induced a mutation rate that was still detectable by the present methods in samples of the size tested. One set of controls was run for all four treated lots, the control eggs being kept in the radiation chamber for 32 minutes but shielded from the ultraviolet light.

After the treated eggs developed into adults, the males were selected and tested for second chromosome lethals by MULLER'S sifter technique (ALTENBURG and ALTENBURG 1952). One of the second chromosomes contained speck (*sp*) as a marker and the other one non-speck (+).

RESULTS

It will be seen from table 1 that the mutation rate relative to the dose of ultraviolet increases rapidly in going from dose 1 to dose 2 ($1.4 \pm .5\%$ to $7.9 \pm 2.5\%$, respectively). Thus a doubling of the dose causes more than a doubling of the rate. Therefore the present data indicate that the rate is an exponential function of the dose, at the lower doses employed. At the higher doses the rate tapers off ($8.6 \pm 2.3\%$ for dose 3 and $5.2 \pm 2.0\%$ for dose 4). The difference in rates for doses 3 and 4 is not significant, and it is not possible to tell from the data how the rate is varying with the dosage at these higher doses. However, it is not probable that an increase in dose actually causes a falling off of the rate in going from dose 3 to 4, since previous work has shown that the curve continues upward in this dosage range.

In view of the relatively great variation in mutation rates induced at different times by ultraviolet of approximately the same dose, it is realized that many intangible conditions influence the ultraviolet induced mutation rate,

TABLE 1

Mutation rates induced in Drosophila by relatively low doses of ultraviolet light

Distance	Duration	Dose μw-sec/cm²	Relative dose	No. ♂♂	No. chromosomes	No. lethals	Mutation rate	Induced mutation rate
360 cm	8 min.	880	1	25	1,005	18	1.8 ± 0.5%	1.4 ± 0.5%
" "	16 "	1760	2	27	1,341	111	8.3 ± 2.5	7.9 ± 2.5
" "	24 "	2640	3	21	800	72	9.0 ± 2.3	8.6 ± 2.3
" "	32 "	3520	4	19	904	50	5.6 ± 2.0	5.2 ± 2.0
Controls				26	1,031	4	.4 ± 0%	

and that therefore too much confidence cannot be placed in the exact rates observed in the present experiments. Obviously a large scale experiment would be desirable, concentrating on the two low treatments (8 and 16 minutes) and possibly with the addition of a dose intermediate between these two. However, it should be pointed out here that in an earlier experiment (ALTENBURG and ALTENBURG 1952) a dose of ultraviolet about equal to the present dose 1 gave a comparable mutation rate ($1.9 \pm 0.8\%$, dose 836 μw-sec/cm², 1,252 tested chromosomes).

Figure 1 gives the dose–rate curve based for the most part on the present experiments but including also one point based on the previous experiment just mentioned.

DISCUSSION

The steepness of the dose–rate curve at the lower doses in the present experiments indicates that the primary mutagenic action of ultraviolet is a multiple hit effect and that at the higher doses certain factors, possibly physiological, not present at the lower doses, are masking the primary effect. How-

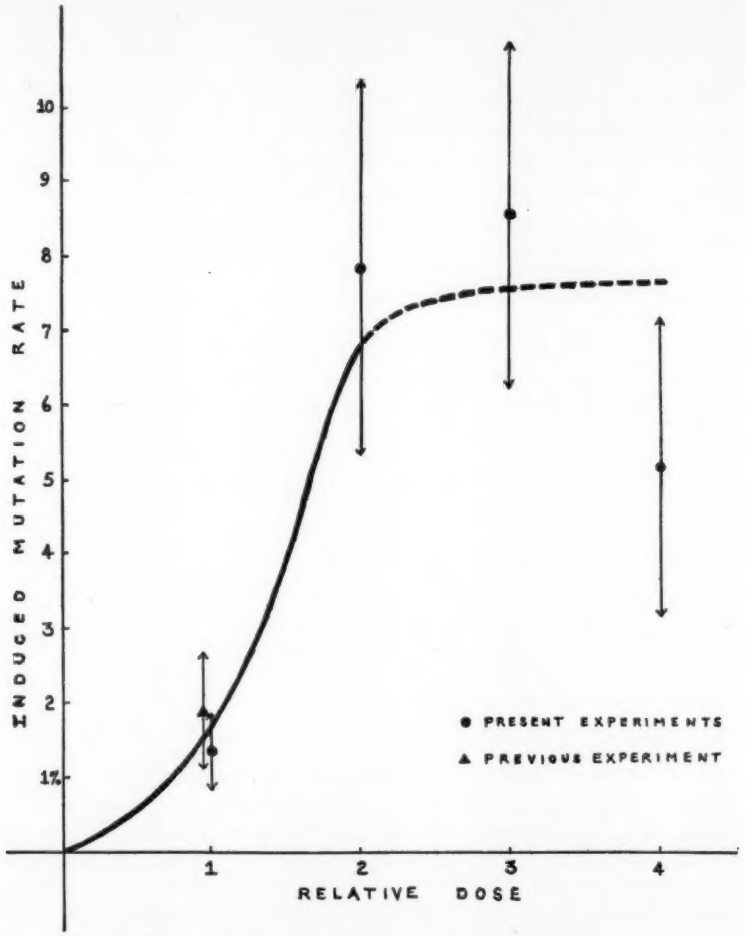

FIGURE 1.—Curve showing variation of mutation rate with increase in dose at low doses of ultraviolet light (actual dose = relative dose × 880 μw-sec/cm^2).

ever, it is also possible that the mutagenic action of ultraviolet is largely indirect and depends in part at least on the production of mutagenic substances which perhaps are reversibly changed or destroyed as fast as they are produced at the higher doses.

SUMMARY

The experiments herein reported indicate that the mutation rate induced in Drosophila by low doses of ultraviolet light is an exponential function of the

dose. Thus, a doubling of the dose at the lower dosage range (from 880 μw-sec/cm^2 to 1760 μw-sec/cm^2) was found to cause an increase in rate from 1.4 $\pm$ .5% (1,005 tested chromosomes) to 7.9 $\pm$ 2.5% (1,341 tested chromosomes). Further increase in dose was found to cause relatively little increase in mutation rate. Thus for a dose of 2,640 μw-sec/cm^2 the rate was 8.6 $\pm$ 2.3% (800 chromosomes), and for a dose of 3,520 μw-sec/cm^2 the rate was 5.2 $\pm$ 2.0% (904 chromosomes). This would indicate that possibly the primary effect of ultraviolet on the mutation rate is a multiple hit effect but is masked at the higher doses by factors not operating at the lower, or operating to a lesser extent. A second possibility is that the mutagenic action of ultraviolet is indirect and depends on substances which are reversibly changed or destroyed as fast as they are produced at the higher doses.

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ABSTRACTS OF PAPERS PRESENTED AT THE 1952 MEETINGS OF THE GENETICS SOCIETY OF AMERICA

ITHACA, NEW YORK, SEPTEMBER 8-10, 1952

ALEXANDER, MARY L.*, Biology Division, Oak Ridge National Laboratory, Oak Ridge, Tennessee. X-ray induced mutation rates at specific loci on the third chromosome of Drosophila melanogaster. — Mutation rates at eight specific loci in the third chromosome of Drosophila melanogaster were obtained both for irradiated spermatogonia and mature sperm. The general procedure was designed to parallel studies in the mouse in which mutation rates at specific autosomal loci have been determined for irradiation of spermatogonia. Special techniques were employed (1) to limit the sperm sample from treated adult males, (2) to detect "spontaneous cluster" mutations, (3) to assure samples from treated spermatogonia by use of early larval stages. — Wild-type males treated as larvae with 900 r of X radiation were mated, individually, to several homozygous ru h th st p^p cu sr e^s females. The F_1 offspring were examined for mutations. Stocks of the recovered mutants were established and the loci involved were rechecked by making crossover tests. One single and three mutant clusters, which gave ratios varying from one mutant per 15 sperm to one mutant per 119.6 sperm, were obtained from 656 males treated as larvae. The mutation rate, calculated by appropriate weighting, is 2.65×10^{-8} per roentgen per locus. — Mature males were treated with 3000 r of X radiation and pair mated to females homozygous for the above-mentioned markers. A total of 68 mutations were recovered from a sample of 49,521 to give an average rate of 5.72×10^{-8} per roentgen per locus for the eight loci. The rates per locus varied from 1.35 to 8.75×10^{-8} per roentgen. The induced mutations from treated sperm have been lethal, semilethal, viable, or phenotypically normal when tested in the homozygous condition. No mutations have been observed in unirradiated sperm. A12.

ALTENBURG, E., J. BERGENDAHL and L. S. ALTENBURG, Rice Institute, Houston, Texas. The failure of a nineteen-fold difference in intensity of ultraviolet to influence the mutation rate in Drosophila. — The polar caps of two different lots of Drosophila eggs were treated with ultraviolet from a germicidal lamp, each lot receiving the same total dose of ultraviolet (about 3,800 μw-sec/cm.^{2}), the one lot however receiving ten times as intense ultraviolet as the other in one series of experiments and nineteen times in another series. Lethals induced in the second chromosome were looked for by Muller's sifter technique. No significant difference was found in the lethal rates induced by the two intensities in either series considered by itself or in the two combined. E7.

*AEC Postdoctoral Fellow.

ATWOOD, K. C., Oak Ridge National Laboratory, Oak Ridge, Tennessee. Different actions of ultraviolet and X rays revealed by heterokaryon methods. — Experiments using conidia from Neurospora heterokaryons with appropriate genetic markers can give several kinds of information about the biological action of radiation or other agents. The survival, the apparent proportion of homokaryotic cells, and the proportion of nuclei carrying recessive lethal mutations can be measured simultaneously. The relative values of these variables after treatment with X rays differ at all doses from those obtained with ultraviolet. The most extreme difference is seen in the proportion of homokaryotic cells among the survivors, which, with X rays, increases rapidly with dose, but shows little change with ultraviolet radiation. Other types of experiments strongly suggest that the effective ultraviolet damage in these conidia is largely nuclear. If this is so, the above result can be interpreted to mean that the ultraviolet effect can be reversed by an interaction between the affected and unaffected nuclei, while the X-ray effect cannot be reversed in this manner. E5.

BAKER, WILLIAM K., Biology Division, Oak Ridge National Laboratory, Oak Ridge, Tennessee. Position effects of a gene normally located in heterochromatin. — The closest known gene to the centromere of chromosome 5 in D. virilis is peach. Fifteen different R(+) rearrangements which produce variegated eyes when heterozygous with pe have been analyzed genetically and cytologically. In nine of these cases, one or more breaks are detectable immediately adjacent to or in the few bands of heterochromatin which comprise the proximal end of chromosome 5. In the remaining cases genetic evidence shows the presence of a translocation involving chromosome 5 but the rearrangement is not cytologically discernible (presumably both breaks in basal heterochromatic regions). In one of the latter cases (Y;5 translocation) the break in 5 is known from genetic evidence to be distal to the peach locus but no chromosome abnormality is detectable. The above facts indicate that the peach locus is normally located in heterochromatin. — The following variations in the euchromatic-heterochromatic relationship will produce variegation: foreign heterochromatin brought distal to the peach locus, euchromatin placed either proximal or distal to the locus. Although no cases have been demonstrated cytologically in which variegation is produced by foreign heterochromatin brought proximal to the locus, their occurrence might be inferred from other evidence. — It is obvious from these data that the relationship between heterochromatin and variegation is not as simple as previous workers have proposed. The high frequency with which peach variegation is induced by X rays (about one out of every 30 sperm receiving 5000 r carry a rearrangement causing variegation) indicates that almost any rearrangement of the basal heterochromatin of chromosome 5 is sufficient to alter the action of the pe gene in somatic tissue. L10.

BAKER, WILLIAM K., and ELIZABETH S. VON HALLE, Biology Division, Oak Ridge National Laboratory, Oak Ridge, Tenn. The effect of oxygen concentration on the induction of dominant lethals. — The production of dominant lethals in males of D. melanogaster was studied as a function of X-ray dosage and oxygen concentration (air, 5% oxygen and nitrogen). The experiments were designed so that the dominant lethals induced in sperm which were ejaculated in copulation with single females during the first 24-hour period after irradiation could be separated from lethals induced in sperm used during the second 24-hour period. Dominant lethals were determined from counts of hatched and unhatched eggs laid by these females. — Irradiating males in nitrogen effectively reduces, as compared to air, the dosage by a factor of 1.5 in the first sperm batch but by only 1.2 in the second batch. Thus the end result of oxygen action is less in the case of dominant lethals than that reported for recessive lethals and chromosome aberrations. The further decrease in oxygen effect in the second sperm batch is due solely to a reduction in number of dominant lethals recovered in males treated in air and 5% oxygen. The number induced in nitrogen-treated flies remains constant over the two-day period. — It is generally agreed that dominant lethals induced in sperm are caused mainly by chromosome breakage with the resulting formation of inviable rearrangements. These data can best be interpreted on the view that the number of breaks induced by X rays is independent of oxygen concentration but that the broken ends formed in nitrogen are more likely to rejoin (either to restitute or to form new arrangements) than those induced in air. A8.

BARRATT, R. W., DOROTHY L. NEWMEYER, and D. D. PERKINS, Stanford University, Calif. Map construction from Neurospora tetrads, and the determination of confidence limits. — Neurospora tetrad data have been compiled from published and unpublished sources. Since the majority of crosses involve only one gene locus and centromere, or two gene loci, the latter have been classified into ditype segregations (parental + non-parental), which are equivalent to 1st division segregations, and tetratypes, which are equivalent to 2nd division segregations. Data are thus treated consistently whether from intervals bounded by one gene locus and centromere or from intervals bounded by two genes, but at the expense of scoring 4-strand double crossover products as non-recombinants. The resultant maps would be entirely valid only if interference were complete within each interval, (See abstract, Perkins, Barratt, and Newmeyer). — Confidence limits were determined at the 5% level for tetratype or 2nd division segregation frequencies from zero to 0.5, and a family of curves constructed for populations up to 600 asci. Upper limits for zero tetratypes were calculated directly from the appropriate binomial term, and limits for frequencies from 0.01 to 0.5 were obtained using binomial probability paper (Mosteller and Tukey 1949). Data have been adequate for mapping 70 loci, dis-

tributed in the seven linkage groups. Maps have been constructed and confidence limits indicated. G11.

BERRY, MARION E. and H. H. PLOUGH, Amherst College, Amherst, Mass. Transduction of multiple nutritional requirements in Salmonella typhimurium. — Auxotrophic mutants of Salmonella typhimurium having multiple nutritional requirements were studied to determine the ability of transduction of these multiple nutritional traits. Every test utilized two strains having different requirements. Each strain was grown in the sterile filtrate of the other strain; subsequently, the progeny were plated on media selective for new combinations of biochemical requirements. Not all strains are necessarily susceptible to the activity of a given filtrate. It was found that the multiple requirements of one strain may be transduced singly, or in any combination of two or more, to another susceptible strain. Not only the requirements, per se, but also the wild-type alleles of the recipient cell genes present in the donor strain may also be transduced in this manner. These traits act independently and appear to be assorted at random among the recipient cells. T1.

BLOUNT, JERRY L., Northwestern University, Evanston, Ill. Structural and behavioral alterations of chromosomes of Drosophila treated with acetic acid.* A variety of genetic effects are induced in D. melanogaster treated with vapors of glacial acetic acid at various stages of development. Among the induced somatic changes in chromosome structure and behavior are non-disjunction, disjunction, crossing over, deletion and possibly mutation. Eggs, second and third instar larvae, and early pupal stages were treated with the acetic acid vapor for varying periods of time. The most striking result is a delayed effect, significantly increasing the frequencies of somatic non-disjunction and crossing over of the X-chromosome in the F_1 of treated individuals. Germinal non-disjunction also is increased significantly, but to a smaller extent than the delayed somatic effects. Several types of somatic spots unexpected on the basis of crossing over or non-disjunction may be accounted for in terms of mutation or deletion. In view of a number of Minute mutations, both germinal and somatic, obtained with acetic acid treatment, deletion would seem the more probable explanation for these unusual cases. Most of the twin spots produced can be explained best by the occurrence of either two separate but simultaneous events of non-disjunction or somatic segregation (disjunction of homologues). Although delayed somatic crossing over is one effect of acetic acid treatment, the evidence suggests the principal delayed effect to be centromeric, inducing inactivation or inhibition of normal disjunctional properties, or inhibition of normal centromere reduplication. H9.

*Work supported by grants from the Rockefeller Foundation and from the Research Funds Committee of the Graduate School of Northwestern University.

BOOTHROYD, E. R. and B. E. WALKER, McGill University, Montreal, Que., Canada. Somatic chromosome numbers in mice. — Variations in chromosome numbers within somatic tissues of animals and plants have recently received considerable attention. The possibility that aneuploidy is widespread in mammals has been suggested by Therman and Timonen as a result of finding a great range of chromosome numbers in human endometrium prepared by the Feulgen squash technique. The present investigation was started to test for the occurrence of aneuploidy in mice. Squash preparations of new-born mouse intestine showed a wide variation in the number of chromosomes per group. Since this could have resulted from fragmentation of diploid complements other techniques are being devised to reduce the amount of cell breakage. One such technique has shown no chromosome groups which deviated from 40 by an amount greater than might be attributed to errors in counting. Work is in progress on other methods for studying chromosome groups in unbroken cells. I5.

von BORSTEL, R. C., University of Pennsylvania, Philadelphia, Pa. Effects of nitrogen mustard on nuclei during meiosis and cleavage of Habrobracon eggs.* — Habrobracon eggs in first meiotic metaphase (metaphase I) were treated with a lethal dose of the nitrogen mustard, methyl bis (beta chloroethyl) amine hydrochloride, and allowed to develop unfertilized. Preliminary analysis reveals no chromosome fragments in the second meiotic metaphase. However, approximately 35% of the second meiotic anaphase and pronuclear stages have fragments or bridges, and nearly all the first and second cleavages show bridges, or fragments resulting from bridges. These data contrast sharply with the results of lethal doses of x-rays in which such chromosome rearrangements are found in nearly 100% of the cases at all stages of meiosis. The increased frequency of chromosome aberrations in later stages produced by nitrogen mustard suggests a predominance of single chromatid breaks and/or a delayed effect of the nitrogen mustard. — Following exposure of metaphase I eggs to a lethal dose, the nuclei continue to divide for several cleavages. About 65% stop in the sixth cleavage and 35% stop in the seventh. Mitosis is arrested in the interphase stage and the nuclei swell to as much as 200 times their normal volume. The nucleolus in each swollen nucleus of these haploid embryos has also enlarged. Unpublished data of A. R. Whiting, showing that nitrogen mustard treated sperm produce similar enlarged cleavage nuclei, indicate that the phenomenon is purely nuclear. When eggs are x-irradiated in metaphase I, nuclear swelling occurs at approximately the same time as with the mustard; swelling is identical with that caused by nitrogen mustard. A2.

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BREWBAKER, J. L. and W. P. ANDERSON, Cornell University, Ithaca, N. Y. Multiple alleles conditioning leaf markings in Trifolium repens. — White leaf markings in T. repens are genetically controlled by a series of multiple alleles. All combinations of the following seven alleles have been studied; V^h ('high' full V); V^l ('low' full V); V^b (broken V); V^{by} (broken V with yellow tip); V^{fi} ('filled in' V); v^P (V-tip); and v (absence of marking). The ten compounds of the first five alleles exhibit mosaic dominance patterns, i.e., the patterns of each allele are expressed in the heterozygote. The v^P allele is dominant to the v allele, and recessive to all other alleles. The v allele is completely recessive to all other alleles. Although the phenotypic expressions of the alleles vary slightly with regard to age and shape of leaf as well as to cultural conditions of the plant, the relative positions of the different markings on the leaf are quite consistent. — The V locus exhibits no linkage with the S (incompatibility) or M (red midrib) loci. Compounds expressing mosaic dominance have been obtained in autotetraploids of T. repens and in the amphidiploid hybrid, 4N T. repens X 4N T. nigrescens. — The principal data from these investigations will be presented and clones exhibiting the phenotypes of the alleles singly and in combination will be demonstrated. D1.

BRIGGS, R. and T. J. KING, Institute for Cancer Research and Lankenau Hospital Research Institute, Philadelphia, Pa. Transplantation of embryonic nuclei into enucleated frogs' eggs, and the problem of nuclear differentiation during development. — The problem of whether nuclei remain identical during embryonic development, or become differentiated as the various parts of the embryo differentiate, has remained unsolved because of the lack of an adequate technique for testing embryonic nuclei. Such tests could be obtained by transplanting nuclei from various parts of the embryo back into enucleated eggs, the development of which would then indicate whether or not irreversible nuclear differentiation had occurred. Recently we have developed a method for transplanting nuclei from advanced blastula cells into enucleated Rana pipiens eggs (Briggs, R. and T. J. King, in press, Proc. Nat. Acad. Sci.). When the nucleus is from the same species as the egg cytoplasm the egg cleaves and can develop into a normal embryo. When the nucleus is from a different species (R. catesbeiana) the enucleated pipiens egg which receives it forms a blastula which is then arrested and subsequently dies, exactly as do the normally produced hybrids between the two species. These and other experiments prove that the blastula cell nucleus can be transplanted in undamaged condition. The details of the method and its application to the problem of nuclear differentiation will be discussed. I4.

BRILES, W. E., Texas A. & M. College System, College Station, Texas. The drift of a series of blood group alleles in related inbred lines of chickens. — In the development of three inbred lines from a single population of White Leghorns considerable gene drift has oc-

curred at the blood group locus B. Studies on these three lines reveal that at least five alleles were present in the original population. Although there has been no conscious selection for or against any of the B alleles, all three of the lines possess two alleles in common while each line possesses a third allele not shared with either of the other two lines. The chicks hatched in the 1952 season (350 or more per line) were tested for the B alleles at four weeks of age. The frequencies of the two common alleles (B^2 and B^7) were, respectively, .26, .64, .33, and .68, .07, .64 in the three lines T2, T3, and T4. The frequencies of each of the three alleles (B^9, B^6 and B^8) present exclusively in the respective lines T2, T3, and T4 were found to be .06, .29, and .03. From these frequencies it appears that one allele in each line may be in danger of extinction as inbreeding is continued in these closed populations. B6.

BRINK, R. A. and R. A. NILAN, University of Wisconsin, Madison, Wisconsin and State College of Washington, Pullman, Washington. The genetic basis of light variegated pericarp in maize. — Evidence has been obtained for the occurrence of a genetic unit termed Modulator which differentiates between two mutable pericarp phenotypes, light and medium variegated. Lights are assumed to carry Modulator, whose effect is to lower the frequency of mutations of variegated (P^{VV}) to self color (P^{RR}). Modulator is transmitted separately from P, and the site which it occupies varies. Medium variegateds lack Modulator but have the capacity to generate it, since a few (ca. 1-5 percent) of their offspring are lights. A self colored mutant patch on a medium variegated ear frequently is associated with an adjacent light variegated area. Evidently such twinned phenotypes arise from a single mutational event. The latter must involve two different loci since the change from variegated to self color is known to result from a mutation at the P locus and it is now shown that the differential between light and medium variegated (Modulator) is transmitted separately from P. Apparently Modulator is released from the P locus when variegated is transformed to self color, and may be assumed to be the unstably associated pigment-suppressing element in the P^{VV} allele which distinguishes the latter from P^{RR}. When variegated mutates to self color, Modulator may be transposed from the P locus to one or another site and, when so reduplicated (P^{VV} + Modulator) in the genome, changes the variegated phenotype from medium to light. Il.

BULL, ALICE LOUISE, Yale University, New Haven, Conn. Embryological effects of three second chromosomal deficiencies in Drosophila melanogaster. — A study of three overlapping deficiencies for the vestigial region of the second chromosome of Drosophila melanogaster, $Df(2)vg^B$, $Df(2)vg^C$ and $Df(2)vg^D$ demonstrated all three to be egg lethal when homozygous. The embryos homozygous for the vg^B deficiency are characterized, during early development, by "sticky" mitoses. In the majority of anaphases and telophases, Feulgen positive

strands connect one or more chromosomes. Such bridges can be found in all dividing tissues, until mitoses decrease after about the tenth hour. From this time on pycnosis and tissue disorientation result. Morphologically the homozygotes appear normal until the period following shortening of the embryos. Dorsal closure is never completed and the head does not involute. — The principal effect of the other two chromosomal deficiencies is on the involution of the head. This process fails in the majority of the vg^D lethals, resulting in the mouth armature developing as separate sclerites on the surface of the head. A number of the homozygous vg^C embryos are characterized by abnormal head involution. The mouth hooks fail to come into place and in many cases the foreshortened cephalopharyngeal bars are thickened and warped. In both these types the differentiation of other structures and organs appears to be completed, but the larvae fail to hatch. L1.

BURDICK, A. B., Purdue University, Lafayette, Indiana. Experimental evidence relating to one postulate of the new Russian genetics. — One frequently mentioned principle of Russian genetics is that grafting generates hereditary instability in the scion resulting in progeny differing from the original line. This postulate was tested in 90 tomato grafts among nine lines differing with respect to five Mendelian genes and other quantitative characteristics. Scions were defoliated daily but were allowed to set fruit. — None of the Mendelian characters (fruit skin color, flesh color, pubescence, plant height, leaf shape) was changed in stocks or scions. Fruit size on many scions was smaller than was comparable fruit on ungrafted plants. — The first seed generation from stock and scion of two grafts was grown under both field and green house conditions. The ungrafted parent lines and their sexual hybrid were also grown along with the stock and scion progenies. Field data were obtained on all Mendelian gene differences between the original stock and scion as well as on the polygenic traits of fruit size and days to first ripe fruit. Greenhouse data included plant height, leaf shape, days to anthesis, and dry weight of plant. The expression of all the Mendelian gene differences was the same in stock and scion progenies as in progenies of ungrafted full sibs. Certain noticeable differences in expression did appear in the plant weight and anthesis data but careful statistical scrutiny failed to disclose any significant differences. J2.

BYERS, HELEN L., and H. J. MULLER, Indiana University, Bloomington, Indiana. Influence of ageing at two different temperatures on the spontaneous mutation rate in mature spermatozoa of Drosophila melanogaster.* — Although earlier work demonstrated that higher temperature applied throughout life increases the mutation rate, the possible dependence of this result upon differences in rate and kind of

*This work is being supported by a grant from the Atomic Energy Commission.

cell multiplication, growth, and metabolism remains unclear. Experiments were accordingly undertaken to study the influence of temperature on mutation rate in mature spermatozoa since in this stage no growth is occurring and the genes are functionally inactive. — Females were inseminated by males which had engaged in previous copulations. The spermatozoa in these females constituted a group with a minimal frequency of mutations which had occurred before copulation (Muller, these Records, 1946). The inseminated females were then aged three weeks, at 7°C and 27°C. Sugar-carragar medium was employed during ageing to inhibit egg production and thus conserve sperm until later. Offspring from eggs laid after that time were tested for recessive sex-linked lethals. Special stocks, containing crossover suppressors and suitable markers, were used. — Among 3490 chromosomes aged at 27°C, 14 lethals were obtained, a rate of 0.401%, while among 8312 chromosomes aged at 7°C, 14 lethals were obtained, a rate of 0.168%. The difference (0.233%) is 2.38 times its standard error (0.098%). The chance of such a result in the given direction if the actual rates were equal is only .01. Additional data for these temperatures and an intermediate one, now being obtained, will be reported. The present results seem to indicate that thermal agitation itself, apart from its influences on growth and ordinary metabolic reactions, is an important determining factor in spontaneous mutation. L3.

CALDECOTT, R. S., E. F. FROLIK and ROSALIND M. MORRIS, University of Nebraska, Lincoln. Differences in the effects of x-rays and thermal neutrons on dormant seeds of barley. — Dormant seeds of Himalaya barley subjected to 5,000; 10,000; 15,000 and 20,000 r units of x-radiation were compared with seeds exposed to thermal neutrons for 3, 6, 9 and 12 hours at a flux of 4.65×10^8 $N_{th}/cm^2/sec$. — Seeds subjected to the three highest doses of x-rays gave a wide range in height distributions at 14 days and the range increased with the dosage. These differences were significantly greater than the control. With thermal neutrons the height distributions were as narrow for all treatments as for the control. — "Differential" delayed killing characterized the material subjected to the three higher doses of x-radiation. Similar lethality patterns did not occur in the thermal neutron treated material. After 40 days most seedlings in the 12 hour treatment had ceased growing, whereas, all seedlings in the 9 hour treatment were actively growing. — Six hours in the thermal column caused a slightly higher chromatinic bridge frequency in root tip cells than previously had been obtained with a lethal dose of x-rays. However, seedling injury from this treatment was not excessive. The 9 and 12 hour treatments gave an aberration frequency $1\frac{1}{2}$ and 2 times as great, respectively, as has been obtained with a lethal dose of x-rays. — For similar frequencies of chromosomal aberrations, there was less injury in the seeds subjected to thermal neutrons than there was in the seeds treated with x-rays. This indicates that thermal neutrons had a proportionately greater effect on the chromatin than on other cell constitu-

ents. Apparently, much x-ray induced seedling injury is extra-chromosomal in nature. I2.

CARSON, H. L. and W. C. BLIGHT, Washington University, St. Louis, Mo. Sex chromosome polymorphism in a population of Drosophila americana. — From a collection made at a single locality by the Meramec River, Jefferson Co., Missouri, 25 laboratory strains were established; each was derived from a single female which had been inseminated in nature. From each strain, one virgin female and one male were selected at random and crossed to flies of a structurally homozygous stock of D. a. americana. From each female, combination brain-salivary smears (from the same larva) were made of 7 F_1 female larvae. Of 25 females so tested, 13 had the metaphase configuration of D. a. americana and were homozygous for the Xabc gene arrangement; 2 had the D. americana texana metaphase and were homozygous Xab; 10 had structurally hybrid metaphases and were Xabc/Xab. From the test crosses of the latter 10 females, 164 brain-salivary smears of F_1 female larvae were examined; these 10 females transmitted 86 americana-type X chromosomes (V-shaped, fused X-4) all of which were Xabc, and 78 texana-type X's (rod-shaped) which were all Xab. Thus no crossing over between the X inversion and the centromere was observed. Of the 25 males tested, 16 transmitted an americana-type X with the Xabc arrangement, and 9 transmitted a texana-type X which was Xab. The study of these 25 strains, including additional observations on ovary smears from females taken at random from the strains, shows that at least 14 strains have segregating structural hybridity in metaphase shape and correlated gene arrangement in the X chromosome. As each strain was derived from a single naturally-inseminated wild female, these data prove that a high degree of interbreeding between americana- and texana-type flies had occurred in the natural population. B3.

CASPARI, ERNST and ROBERT W. SANTWAY, Wesleyan University, Middletown, Conn. Differences in mitochondria between different strains of the mouse.* — Mitochondria from mouse livers and kidneys were isolated by centrifugation at 24,000 g. Males from the strains C57 B1, BALB/Ci and "isogenic" BALB/Ci containing the genes T/+ and Fu/+ were used. Differences between the mitochondria contained in the livers of these strains were found by chemical and serological methods. As a chemical characteristic, the nitrogen/phosphorus ratio was studied. In the serological experiments, rabbits were immunized with mitochondria and their sera tested with a quantitative complement fixation method. In these experiments, the same serum was tested against mitochondrial antigens from different strains which had been

*This investigation was supported by a research grant from the Division of Research Grants of the National Institutes of Health, Public Health Service.

adjusted to contain equal amounts of nitrogen. BALB/Ci Fu/+ mitochondria differ from those from +/+ and T/+ animals by their higher anticomplementary activity. BALB/Ci T/+ mitochondria react at a higher titer with T/+ antisera than +/+ antigen. Similarly, antigenic differences between BALB/Ci and C57 Bl mitochondria have been found. The $\frac{N}{P}$ ratio of liver mitochondria from BALB/Ci is about 10% higher than that of C57 Bl mitochondria. Different mitochondrial fractions have different $\frac{N}{P}$ ratios. It could be established that the difference between BALB/Ci and C57 Bl mitochondria is not due to different relative amounts present in different fractions, but the difference is restricted to the heaviest mitochondria. No similar difference could be found for mitochondria prepared from kidneys. The chemical composition of mitochondria seems to be variable under the influence of genetic as well as developmental factors. H5.

CHASE, H. B. and W. MONTAGNA, Brown University, Providence, Rhode Island. The development and consequences of hairlessness in the mouse. — In hairlessness (hr hr) the first pelage grows normally but falls out at the end of growth without a normal club being formed. The naked skin then becomes progressively abnormal, with hypertrophied cells in the epidermis, and sebaceous and keratinized cysts in the dermis and adipose layers. In the normal hair growth cycle, catagen is the 2-day transition period between active anagen and resting telogen. A club is formed and the bulb everts to form an epithelial strand between the club region and dermal papilla. The base of the strand is the germ for the next hair generation. By telogen the whole follicle, including the strand, is shorter, causing the germ and papilla to lie well up in the dermis rather than in the adipose layer. In hairlessness a catagen strand is formed, but keratinization ceases before a normal club is produced and the strand fails to shorten. The strand becomes attenuated in places then breaks. The result is a sebaceous gland with a tab of external sheath cells in the dermis, and a disconnected germ with dermal papilla isolated in the adipose layer. Usually more than one break occurs and isolated segments of the strand are found. The isolated germ often forms an abortive hair. The tab and the isolated portions, including the germ-papilla, still have the potentialities of external sheath cells for undergoing sebaceous and keratinizing transformations. With the broken continuity of the follicle, however, the consequences are large numbers of sebaceous and keratinized cysts. C7.

CHASE, SHERRET S., Iowa State College, Ames, Iowa. Selection for parthenogenesis and monoploid fertility in maize. — The hypothesis that monoploid-derived stocks of maize would prove particularly rich sources of reproductively successful monoploids appears to be valid. The six single cross hybrids of four monoploid-derived autodiploids

and several double cross hybrids combining these singles were tested for monoploid frequency (rate of parthenogenesis) and for the self fertility of the monoploids obtained. The original stock, the Stiff Stalk Synthetic dent corn, from which the parental autodiploids were derived, yielded monoploids at the rate of 1.3 per thousand seedlings in genetically marked progenies. 9.4% of monoploids from the synthetic set self seed. The group of single cross hybrids yielded monoploids at the rate of 4.3 per thousand, the range being from a low of 1.3 to a high of 7.3. 33% of the monoploids obtained set self seed. In the group of double cross hybrids monoploids were obtained at a rate of 2.9 per thousand with 30.5% of these being self fertile. The above data indicate that a very material increase in the efficiency of the monoploid method for developing homozygous lines of maize can be effected through the development and use of monoploid-derived stocks, such as synthetic varieties. It appears likely that combinations of almost any group of monoploid-derived autodiploids will provide more favorable source stocks and that combination of autodiploids selected for high rates of parthenogenesis and fertility of derived monoploids will provide stocks particularly favorable for the monoploid method. M1.

CLARK, A. M. and W. C. BEISER, University of Delaware, Newark, Delaware. Differential sensitivity of haploid and diploid embryos of Habrobracon to the nitrogen mustards. — Haploid and diploid embryos while in cleavage were immersed in a nitrogen mustard solution of known concentration buffered to pH 7. The compounds tested were methyl bis-(β-chloroethylamine)HCl, ethyl bis-(β-chloroethylamine) HCl, 2,2′ dichlorodiethylamine, and 2,2′,2″ trichlorotriethylamine. These compounds have been shown by other investigators to be mutagenic on other organisms. In all cases, the haploid embryos treated during cleavage were found to be more resistant than comparable diploids. More detailed studies using methyl bis-(β-chloroethylamine) HCl showed that embryos treated during cleavage were either arrested at this stage or proceeded to develop normally into adults. Cytological observations revealed that enlarged nuclei and nucleoli occurred, arrested at interphase. Exposure of older embryos resulted in normal hatching, but post-embryonic injury was observed. These results are identical to those obtained by use of x-rays in similar experiments on the same organism. A3.

COHEN, CARL and J. L. FULLER, R. B. Jackson Memorial Laboratory, Bar Harbor, Maine. The inheritance of blood types in the dog. — Four isoantigens (A, B, C, D) occurring on the dog red cell have been described by Young et al. An analysis has been made of Young's data on 384 dogs of unrecorded ancestry to determine if there was evidence that any of these isoantigens were derived from allelic genes. In addition, serological studies have been carried out on purebred dogs of the Jax colony. In some cases determinations of blood type have been made on four consecutive generations. — Purebred Basenjis have

yielded types A, B, C and D; our sample of Cocker Spaniels has not shown D. Because of the limited source of sampling, these results cannot be accepted as characteristic of the entire breed. The genetic evidence indicates that each isoantigen is determined by a single corresponding dominant gene. There is no evidence for linkage nor for the existence of any allelic pairs, although the latter possibility has not been definitely excluded for the A, D pair. — The authors are indebted to Dr. L. E. Young of the University of Rochester School of Medicine and Dentistry for sera used in these determinations. C1.

CONGER, ALAN D. and LUCILE M. FAIRCHILD, Biology Division, Oak Ridge National Laboratory, Oak Ridge, Tennessee. Breakage of chromosomes by oxygen. — Chromosomes are broken in the flower buds and dry pollen grains of Tradescantia by exposure to oxygen concentrations greater than air; the aberrations are indistinguishable from those caused by ionizing radiations. Oxygen treatment of flower buds has broken chromosomes in prophase and resting stage of mitosis and meiosis. In the dry pollen grains, the quantitative relation of breakage to increasing oxygen concentration, pressure, and time of exposure, to temperature, cellular moisture content, and ozone concentration have been measured, as well as various qualitative features. The data show that it is not molecular oxygen which breaks chromosomes, but rather the reaction is caused by a physiological response of the cell itself to increased oxygen concentration. The conclusion that chromosomes are broken by a physical process with radiation, a biological process with oxygen, is clear from the difference in effect that variations in environment (temperature, moisture) have on the two reactions. — The experimental information is compatible with the idea that oxygen causes chromosome breakage by the acceleration of an oxidative (terminal?) enzyme system which produces reactive agents (OH, HO_2, possibly H_2O_2) as by-products. A9.

DAWSON, W. M., T. S. YAO and A. C. COOK, United States Department of Agriculture, Washington, D. C. Heritability of growth, beef characters, and body measurements in Milking Shorthorn Steers. — Heritability estimates were obtained for five growth characters, three beef characters and 19 body measurements from 62 Milking Shorthorn steers raised at the Agricultural Research Center, Beltsville, Md., from 1943 through 1949. Component variances for each character were computed. Variance due to years and variance due to years x sires interaction where the latter was positive were considered as caused by the environment and eliminated. Sires variances were adjusted by subtracting the years x sires interaction where the latter was negative on the assumption that a negative interaction indicated that a considerable environmental influence of years was included in the sire variance. The paternal half sib method was used to estimate the heritability. Estimates of the percent heritability of the 27 characters were by groups: Growth measures—birthweight 16.3 $\pm$ 15.0, days to weaning

43.9 ± 18.0, days to final weight 900 lbs. 3.2 ± 13.4, efficiency of gain 17.2 ± 15.2, average daily gain 43.6 ± 0.2; beef characters—slaughter grade 61.6 ± 19.6, carcass grade 56.6 ± 19.2, dressing percentage 69.9 ± 20.3; body measurements—height at withers 63.2 ± 19.8, height at floor of chest 1.3 ± 13.2, height at flank 55.3 ± 19.1, depth of chest 0, length of body 0, length of rump 0, length of coupling 12.3 ± 14.8, length of nose 0, width between eyes 37.6 ± 17.4, width of muzzle 50.1 ± 18.6, width at shoulders 0, width of chest 4.2 ± 13.6, width at last rib 0, width at loin 0, width at hip 6.2 ± 13.8, circumference foreflank 26.4 ± 16.2, circumference navel 54.8 ± 1.9, circumference rear flank 27.5 ± 16.3, and circumference shin bone 0. B12.

DeLAMATER, E. D., University of Pennsylvania, Philadelphia, Pa. The nuclear cytology of Escherichia coli during mitosis. — Observations on Escherichia coli stained and processed by the freezing-dehydration procedures previously described by the author, indicate that this organism, which is much smaller than Bacillus megatherium, follows the same pattern of nuclear division as has been previously described in Bacillus megatherium and other bacteria. The prophase chromosomal contraction, the metaphase, which is characterized by the presence of polar centrioles, and the anaphase and telophase stages appear to be entirely similar to those already described, though smaller. Cytologically the chromosome number in Escherichia coli, K-12, appears to be 3 in the vegetative phase. Photographic evidence is presented in support of these observations. H8.

DeLAMATER, E. D. and M. E. HUNTER, University of Pennsylvania, Philadelphia, Pa. A further study of the chromosomes of Bacillus megatherium during vegetative mitosis, with particular emphasis on the prophase stages. — Previously presented evidence indicates that a true mitotic process occurs in Bacillus megatherium. The preparations currently studied were made by the staining and freezing-dehydration techniques already described, but were crushed at the time of mounting. In such smear preparations the following observations were made. — The chromosomes of the interphase nucleus appear as single, very delicate, tangled threads. During prophase a progressive contraction and spiraling occurs. The chromosomes appear to undergo stages typical of leptotene, pachytene, diplotene and diakinesis. The chromonemata become visible as paired threads during early pachytene. The paired chromonemata have corresponding bands which appear to be equivalent to chromomeres. Spiraling of the chromonemata is likewise demonstrable. The chromosomes further contract to form diplotene loops, and subsequently the two chromonemata appear to separate completely except at the point of common attachment to the centromere. The compact chromosomes become oriented on the metaphase spindle between two centrioles, at which time each chromosome is observed to be double. Chromosomal separation appears to occur at the time of division of the centromere, when anaphase is initiated. Following complete

separation of the chromosomes in anaphase, the subsequent telophase stages occur with the concomitant reformation of the nuclear membrane and the elongation of the chromosomes by uncoiling. The current evidence indicates clearly the equivalence between the chromosomes and the mitotic process of Bacillus megatherium and that of larger organisms. Further, the chromosomes can be fully characterized and recognized. H7.

DOERMANN, A. H. and M. B. HILL, Biology Division, Oak Ridge National Laboratory, Oak Ridge, Tenn. The arrangement of genetic loci in bacteriophage T4. — Turbidity of plaque halos in bacteriophage T4 is influenced by previously undescribed genetic factors. Five of these factors at separate loci have been studied. All two-factor crosses have been made within a set of nine genetic loci consisting of the five tu loci and four genetic factors of previously described types. The recombination data indicate that these nine loci fall into three linkage groups containing one, three, and five members respectively. The data, furthermore, answer two questions: (1) are the loci arranged in linear order; (2) is recombination in one region statistically independent of recombination in an adjoining region. The results show that a linear arrangement of the loci is compatible with the data in both linkage groups. This is clear, since it is invariably true that when the loci are arranged in the appropriate order, the recombination value for any two nonadjacent loci is greater than the value for any pair of intermediate loci. Concerning the second question, it may be said that the data show a slight positive correlation in the occurrence of simultaneous recombinations in neighboring regions. Although at first sight this "negative interference" is surprising, one can logically account for it by including random repetitive mating in the model of phage reproduction. Visconti and Delbrück have recently formulated such a model. Application of the parameters determined by them from independent experiments destroys this correlation, indicating that recombinations in individual matings are independent of one another. K2.

DUNN, L. C. and S. GLUECKSOHN-WAELSCH, Columbia University, New York. The genetic behavior of seven new mutant alleles which arose in one balanced lethal line in the house mouse. — The balanced line #29 bred in our laboratory for 20 years normally produces three classes of zygotes: T/T, abnormal embryos which die at about ten days after fertilization; t^1/t^1, embryos which die before implantation; and T/t^1, which survive as tailless individuals to perpetuate the line. Occasionally normal-tailed animals are found as exceptions. Seven such exceptions studied in detail proved to be t^1/t^n and each has given rise to a new tailless line as follows: Line 3—T/t^3 tailless; t^3/t^3 viable; normal tail; Line 4—T/t^4 tailless; t^4/t^4 lethal at 8 days after fertilization; Line 7—T/t^7 tailless; t^7/t^7 viable; normal tail; Line 8—T/t^8 tailless; t^8/t^8 viable; normal tail; Line 9—T/t^9 tailless; t^9/t^9 lethal; 9 days; Line 11—T/t^{11} tailless; t^{11}/t^{11} viable; normal tail;

Line 12—T/t^{12} tailless; t^{12}/t^{12} lethal; stage undetermined. No recombination with T is shown by any of these, or of the older alleles, t^0, t^1, t^2. Each allele is viable in compound with each other one; has distinctive effects on the segregation ratio T : t^n in males and on male fertility in compounds with other alleles, t^x/t^n, which show gradations from sterility through quasi-sterility to nearly normal fertility. The alleles t^0 and t^1 prevent recombination in the region T-Ki (4 map units); t^3 however takes part in recombination in this region. It is thus unlikely that t^3 which arose from t^1 contains an inversion. Other viable and lethal alleles are being tested for these effects (suppression of recombination and inversion). It is assumed that each allele is due to a different mutation in the genetic material at or near Locus T. I3.

DUNN, L. C. and WALTER C. MORGAN, JR., Nevis Biological Station, Columbia University, New York. Mutations at a mutable locus (t) in wild Mus musculus. — Seven males from a population of wild Mus musculus, kindly supplied by Dr. Howard A. Schneider (cf. Schneider, 1946), have been tested for mutations at locus T by mating with short-tailed (Brachy) T/+ females. The presence of t-type mutant gametes from each of four such wild males has been proved by recovery in F_1 of tailless offspring some of which have been tested and shown to be T/t^n. These four wild males tested by T/+ gave 67 normal, 6 Brachy, and 48 tailless, indicating the genotype of each wild male to be $+/t^n$ and a segregation ratio amongst the gametes of the wild males of about $8t^n$: 1+. This excess of mutant sperm is similar to that observed for other t alleles in laboratory stocks and may be in part responsible for the retention of t^n alleles in wild populations. The occurrence of such mutations in normal wild mice makes it improbable that the instability of this locus in the inbred balanced lethal line T/t^1 (Dunn and Gluecksohn-Waelsch, 1951) is due to rearrangements caused by t^1. These alleles may now be used to elucidate the size and breeding structure of wild mouse populations. T2.

EDMONDSON, MARGARET and HELEN U. MEYER, Indiana University, Bloomington, Indiana. Influence of oxygen and of temperature on the rate of sex-linked recessive lethals induced by ultraviolet in the polar cap of Drosophila melanogaster.* — Two lots of embryos were treated simultaneously with uv. from a Germicidal lamp, with "photoreactivating" wave lengths removed, in atmospheres of oxygen and nitrogen, respectively. Both lots were again divided into two groups, placed 5 minutes after treatment at 17°C and 27°C, respectively, and thereafter cultured at these temperatures. In the 5-minute interval the embryos in polar cap stage were chosen. In the main (large-scale) series of the work, the exposure lasted 50 to 60 seconds, delivering the intentionally low dose of 90 to 108 ergs/mm^2. The gases were ad-

*This work was supported by a National Cancer Institute, U. S. Public Health Service research grant.

ministered from 5 minutes preceding until 2 minutes following irradiation. — Daughters of females whose pole cells had been irradiated were scored for recessive sex-linked lethals. The percentages of lethal mutations in the main series, based on 480-560 chromosomes per group, were: controls, 0.19 ± 0.19; oxygen-warm, 2.08 ± 0.82; nitrogen-warm, 2.34 ± 1.24; oxygen-cold, 7.54 ± 2.64; nitrogen-cold, 9.52 ± 3.70. The cold groups, taken together, show a mutation rate which is significantly higher than that of the warm groups, the difference being 2.65 times its standard error ($P < .01$). The data suggest, further, that the treatment in nitrogen was more effective mutagenically than that in oxygen. These results are in agreement with those being reported by Meyer and Muller (these Records), on second chromosome lethals in sons of male embryos of the same material, treated simultaneously. — The above sex-linked lethals were localized by crossover tests. Their relative frequencies along the X-chromosome agreed with previous determinations for lethals induced in mature spermatozoa by ultraviolet and by X-rays. A7.

ERK, FRANK C., Johns Hopkins University, Baltimore, Md. Survival of a translocation in laboratory populations of Drosophila melanogaster. — Competition between a 2,3 translocation, associated with the dominant gene Curly, and its wild-type homologues was studied in special population cages supporting about 6000 individuals. In experiments followed over a four-month period, the gametic frequency of the translocation dropped quickly from an initial 25 percent to a low value at which it persisted; at the termination of the experiment, the values were 0.47 percent and 0.56 percent in duplicate experiments. These values, although low, are significantly greater than expectancy for the translocation, and suggest that Curly had a favorable effect. In control populations, where Curly, itself associated with inversions in chromosome 2, competed with its wild-type homologue, the gametic frequency dropped from 25 percent initially to 3.12 percent and 6.05 percent in duplicate experiments. These values, significantly below expectancy, show that selection acted against the Curly heterozygotes during the term of the experiment. Therefore, the slightly favorable adaptive value of the translocation is very likely due to an interaction of Curly with the translocation. B8.

FABERGÉ, A. C., University of Missouri, Columbia, Missouri. Loss of ring chromosomes produced by X-rays in female Drosophila.* — In principle, important evidence on restitution and on primary X-ray induced breaks can be obtained from ring chromosomes. Bauer's well known experiment consisted in X-raying ring-X males, and determining the depletion of daughters among the offspring. It is assumed that in rods, a high proportion of breaks are followed by simple restitution

*Work supported by a research grant from the National Cancer Institute, National Institute of Health, Public Health Service.

which is undetectable, whereas in rings some of the restitutions occur so as to yield dicentric or else interlocked rings which act as dominant lethals. In an attempt to confirm the validity of this interpretation, a converse experiment was done in which $X^{c2}/M5$ females were X-rayed; in the female, translocations are presumably no longer an important factor. The dose used, 2000 R, reduced fertility by 28% and increased patroclinous males from 0.53 to 4.72% during the 6 day egg laying period. 17,802 offspring were classified to determine the ratio of X^{c2} to M5 chromosomes. Untreated females gave a ratio of 0.87 among female offspring, 1.15 among male offspring, while irradiated females gave 0.73 among female offspring and 0.93 among male offspring. Thus, just as when males are X-rayed, a ring chromosome is more sensitive to radiation than a rod. The mean of the above values is not very different from the ratio of females to males in Bauer's experiment with the same dose. It can probably be concluded that X-rays produce primary chromosome breaks at about the same rate in the two sexes, though their final results are, as is well known, different. E9.

FORD, LEE, Iowa State College, Ames, Iowa. A cytogenetic study of a set of maize monoploid twins. — Monoploid meiosis was studied in detail in a set of maize monoploid twins derived from L289. Out of about fifty monoploid maize plants studied, these twins were the only plants having a sizeable portion of diploid tissue. One of the twin plants was very favorable for anaphase I division counts, an unusual amount of reduction division taking place also. Restitution nuclei and despiralization of the chromosomes in telophase I were observed. Division II counts showed regular reduction of chromatids. Odd "giant cells" were found in one twin plant probably due to tendency of some monoploid cells to fuse together plus disintegration of the cell walls and chromatin. Bridges plus fragments were observed at anaphase I. Both plants were quite favorable for cytological study. M10.

FORSTHOEFEL, PAULINUS F., Ohio State University, Columbus, O. Effect of heterozygosis on litter size in the mouse Mus musculus. — To test for a possible effect of heterozygosity of the young on litter size, inbred female mice were bred to their brothers while sisters were out-crossed. Ten sets of B alb C strain and ten sets of DBA strain mice were mated in this way, two sisters constituting each set. Data involving the DBA mice were insufficient to support a valid statistical analysis because of a high incidence of sterility among the females during the progress of the experiment. Data secured from the B alb C females were more complete and allowed matching of litters of the mice inbred with corresponding litters from their out-crossed sisters. The mean litter size in litters where the young were inbred was 4.82 mice while the mean litter size in litters where the young were hybrids was 6.82 mice. On statistical analysis, the difference of two mice was found to be significant. This difference is ascribable to the heterozygosity of the young since this was the major known dif-

ference between the matched litters. The population seemed uniform within the limits of sampling error. Increased vigor of the hybrid young because of the covering up of recessive deleterious factors received from one inbred parent by dominant normal factors received from the other is advanced as a possible genetic explanation. T3.

FOSTER, M., University of Chicago, Chicago, Ill. Inherited variations in the tyrosinase system in guinea-pig skin. — Studies of tyrosinase and dopa oxidase activities (producing dark pigments) by means of manometric measurements of oxygen consumption of foetal guinea-pig skin homogenates give results indicating no simple parallelism between demonstrable enzyme activity and amount or kind of natural pigment. (1) The genotypes for black (E-aaB-C-F-P-), brown (E-aabbC-F-P-) and pale brown (E-aabbC-F-pp) give approximately equivalent reactions, although brown (--bb--) and pale brown (--bbpp--) hairs show marked and extreme reductions, respectively, of natural pigment as compared with the solid black standard. (2) Skins of genotypes characterized by yellow instead of black pigment show drastically reduced tyrosinase activity. This is true of solid yellow (eeaaB-C-F-P-); of black and yellow spotting (ePePaaB-C-F-P-), in which the reduction in activity is roughly proportional to the relative amount of yellow pigment; and of agouti, or yellow-banded dark hair, types (E-A-B-C-F-P-), at a stage in hair growth when many hair follicles are in the yellow phase of pigment production. (3) Skins of tested genotypes of lower albino series alleles (E-aaB-$c^k c^r$F-P-, or --$c^r c^r$--, or --$c^a c^a$--) show no trace of tyrosinase activity, regardless of intensity of natural dark pigment, genotype --$c^k c^r$-- being approximately as intensely black and --$c^r c^r$-- only slightly less black than the highly active genotype --C--. — The present results confirm in a general way the results of previous dopa reaction studies by Russell and Ginsburg, but strongly accentuate the differences in the second and third groups of above results. (Work supported by a postdoctorate research fellowship from the National Cancer Institute, U. S. Public Health Service.) C3.

FOX, ALLEN S. and NANCY KENT ZIEBUR, Ohio State University, Columbus, Ohio. An attempt to induce specific back-mutation at the inositolless locus in Neurospora crassa by means of antibodies.* — Work of Guyer, of Emerson, and of Anderson-Kottö suggests that antibodies are specific mutagens. To test this possibility, Giles' genetic system for the study of back-mutation of inositolless (i) to wild (i^+) has been used with suitable immunological techniques. A homocaryotic colonial, microconidial, inositolless (col-1, m, fl, i) stock was established from an ascospore produced from Giles' strain 37401-CL30-2a x wild (SY7a-2F8). From a spontaneous reversion of the derived stock,

*Supported by grant from National Institutes of Health, U. S. Public Health Service.

a homocaryotic i^+ stock was secured; the two stocks may be regarded as co-isogenic in other respects. Rabbits were injected intraperitoneally with mycelial extracts of the two stocks. Cross absorption, precipitin tests and microconidial agglutination tests of high-titered antisera failed to demonstrate antibodies specific to i or i^+. In two mutagenic series antiserum-treated i microconidia were plated on minimal medium to detect reversions; normal serum and untreated controls were included. Series I—Microconidia suspended in undiluted antiserum, incubated 24 hours, washed before plating. Total microconidia treated: 39×10^7 with anti-i^+ serum, 2×10^7 with anti-i. No differences between treated and controls were found. 1.12×10^9 microconidia, treated and controls, showed a reversion rate of 3.6×10^{-10}. Series II—Suspensions of microconidia in 1 : 2 antiserum were plated; limited growth occurred because of inositol in the serum. Microconidia treated: 5×10^3 with anti-i^+ serum, 83.6×10^3 with anti-i. Actual number of treated nuclei is unknown, but no reversions were observed. Alternative interpretations are suggested: 1) Antibodies not mutagenic. 2) Antibodies mutagenic, but inactive here because of any of following: a) No specific antibodies in antisera used, b) inositolless locus unresponsive to antibody mutagenesis, c) antibodies fail to reach genes due to membrane impermeability or intracellular destruction. G3.

FRIED, PHYLLIS J. and JOSHUA LEDERBERG, University of Wisconsin, Madison, Wis. Linkage in E. coli K-12. — The distribution of unselected markers among selected prototroph recombinants had implied that the known loci in E. coli strain K-12 were located on one linkage group. Independent verification of the linkage of Lac to M was sought in crosses using other selected markers, so that these were allowed to segregate. In $M^-Lac^+ \times M^+Lac^-$, both M^+ and Lac^- predominated. However, there was no interaction between these factors to verify their linkage to each other. This was corroborated by the non-correlation of the factors M, S, and Th, with P, Lac, and Vl, whereas linkage correlations were found within each of the two groups. — If both selected markers of a cross were confined to one group, the segregation of the other nevertheless deviated markedly from random. (The preferential appearance in the segregants of the alleles contributed by one parent has been associated with the polarity of a recently discovered sex compatibility factor.) — The separation of M and Lac into distinct linkage groups obviates the need for postulating several mutually independent factors linked to M (Lederberg et al. 1951, Cold Spr. Harb. Symp. XVI). T4.

GABELMAN, W. H., University of Wisconsin, Madison, Wisc. Genetic control of the cytoplasmic factor for male-sterility in maize. — The inbred Minnesota A34 contains a single dominant gene capable of converting cytoplasmically male sterile inbreds and hybrids to partially fertile hybrids having only 40 percent aborted pollen. Although 40 percent of the pollen is aborted, anthesis appears normal and these

plants are very similar to normal plants. — F_2 data indicate the presence of modifiers. M2.

GARBER, E. D., Naval Biological Laboratory, Naval Supply Center, Oakland, Cal. "Bent-tail," a dominant, sex-linked mutation in the mouse. — Bent-tail (Bn) is a dominant, sex-linked mutation in the house mouse visibly affecting the structure and length of the tail as well as unknown characters leading to inviability in males and homozygous females. Penetrance is high, at least 95 percent in heterozygous females and 100 percent in males and homozygous females. Homozygous females and Bent-tail males have tails half normal length. Data will be presented indicating that Bent-tail males are hemizygous for the Bn locus which may be in the non-homologous segment of the X-chromosome. C5.

GARBER, E. D., ADELINE J. HACKETT and R. FRANKLIN, Naval Biological Laboratory, Naval Supply Center, Oakland, Cal. The virulence of biochemical mutants of Klebsiella pneumoniae. — Five amino-acid requiring mutants and 1 pyrimidine requiring mutants of K. pneumoniae were as virulent as the parental strain when inoculated into mice either intraperitoneally or intranasally. Three purine requiring mutants were avirulent when inoculated by either route. Virulence was restored with reversion to purine independence. The intraperitoneal inoculation of purine into mice inoculated by the same route with a purine requiring mutant resulted in death. The use of biochemical mutants of pathogens to explore the biochemistry of sites of inoculation or localization in susceptible hosts is suggested. K7.

GARDNER, ELDON J. and GERALD H. STOTT, Utah State Agricultural College, Logan, Utah. Tumorous head genes in combination with modifiers from laboratory stocks and natural populations. — A sex-linked gene (tu-1) located near f and autosomal gene (tu-3) located in the right arm of the third chromosome interact to produce the tumorous head phenotype. A maternal effect is controlled by tu-1. The penetrance is influenced greatly by environmental factors, such as temperature, genetic modifiers, and different alleles of the tumorous head genes. Modifiers of tu-3 have been found in several laboratory stocks and eight natural populations. The main difference between the results of crosses involving flies from natural populations and those reported previously from laboratory stocks was the lack of uniformity among the results of pair matings, suggesting the segregation of genes in the natural populations. The laboratory stocks were apparently homozygous for the modifiers they carried. In contrast, the natural populations carried modifiers in different frequencies. Different alleles of tu-1 were found in three laboratory stocks and one natural population. By combining modifiers with the major genes, a penetrance of nearly 100 percent has been maintained at 25°C. The action of these genes alone and in various combinations with modifiers is under in-

vestigation. Laboratory stocks with phenotypes resembling tumorous head are being studied for the purpose of identifying genetic relationships with tumorous head. L12.

GILES, NORMAN H., JR., Yale University, New Haven, Conn. The effect of a suppressor on allelic inositolless mutants. — Studies have been carried out on a reversion arising by suppressor mutation in ultraviolet-treated microconidia of inositolless mutant 37401. The suppressed mutant grows in the absence of inositol, but at a much slower rate than do inositol-independent wild-type cultures or the usual reverse-mutation types; further, its growth is markedly stimulated by added inositol. Microbiological assays indicate that the mutant is synthesizing inositol. The suppressor combined with the wild-type allele at the inositolless locus produces a culture which, in flask tests, is markedly retarded in growth on minimal compared with wild-type and is not stimulated by added inositol. Genetic tests indicate that the suppressor is not linked with the inositolless locus. The suppressor has been tested for its effect in combination with a series of independently induced, allelic (or pseudoallelic) inositolless mutants (Stanford mutant numbers 37102, 46316, 46802, 64001, 89601 and Johns Hopkins mutant numbers 2626 and 5202). The suppressor appears to be quite specific for mutant 37401, since in no instance has growth in the absence of inositol occurred when the suppressor was combined with any other inositolless mutant. Proof of the actual presence and potential activity of the suppressor in such instances was obtained by its reextraction from such mutants and continued effect in combination with mutant 37401. G2.

GLASS, BENTLEY, The Johns Hopkins University, Baltimore, Md. Dosage curves for sex-linked lethals and dominant Minutes in Drosophila melanogaster females. — Dosage curves for x-ray induced sex-linked lethals have been determined for spermatozoa and oocytes treated simultaneously. Both dosage curves are linear up to 4000r. The frequency of sex-linked lethals induced in ♀♀ per 1000r units (combined data) is 1.55 per 100 chromosomes tested, or 44.4% of the frequency in spermatozoa. — In females few gross chromosomal aberrations are produced, e.g., at 2000r, ♂♂ 224/3542 (6.32%); ♀♀, only 6/2189 (0.27%), in summed series with or without supplementary infrared treatment. It may therefore be concluded that the increase (with dosage) of lethals due solely to point mutations and 1-hit deficiencies is linear in females, and presumably in males also. Since in males chromosomal aberrations are frequently induced by x-rays, and at an exponential frequency with dosage, in spite of which the dosage curve for lethals remains linear, these data may be taken to mean that lethals associated with chromosome rearrangements increase as the frequency of individual breaks increases; hence that position-effect lethals are relatively scarce. — Very consistent dosage curves have been obtained for dominant Minutes induced by x-rays in ♀♀, the fre-

quencies ranging from 0.1% at 500r to 2.4% at 4000r. The curve is not significantly different from a linear increase with dosage. At 3000-4000r, the frequency in spermatozoa is not significantly higher than in oocytes: ♂♂ 53/5832 (0.91%); ♀♀, 98/7743 (1.26%). Inasmuch as most Minutes are deficiencies, this may mean that the decrease in lethal frequency in oocytes, compared with spermatozoa, is due solely to decreased point mutation in females. A4.

GODFREY, GEORGE F. and C. N. WILLIAMS, Oklahoma A. & M. College, Stillwater, Oklahoma. Heritability of growth in the domestic fowl. — Heritability estimates herein reported are based on a selection experiment in which one line of Silver Oklabars was selected for rapid growth, while another line was selected for slow growth as measured by body weight at 6 and 12 weeks of age. Original selections for the two lines were made from the same population. Results of two generations of selection yielded heritability estimates of .19 and .30 for body weight at 6 weeks of age, and estimates of .31 and .32 for body weight at 12 weeks of age. Estimates calculated for males were consistently lower at both ages than those calculated for females. — Selection solely for body weight at 6 and 12 weeks of age resulted in significant differences in adult body weight and egg size between the selected parents of the rapid and slow growth lines. B10.

GOODALE, H. D., Mount Hope Farm, Williamstown, Mass. A study of fine gradations in the breeding of much from little. — During the past 21 years several sublines have been bred from five animals. A small variable white area on the forehead has given rise to maximum, minimal, zero and two random mated lines—one pigmented, the other non-pigmented. The white of some members of the maximum line is 800 times the area of the maximum of the pigmented random mated line, or 4800 mm^2 out of a possible 6000 mm^2. The minimum in this line is about 200 mm^2. White hairs are absent from the forehead of the zero line though present about the muzzle. The members of the minimal line—developed during the past 4 years from the random mated line—mostly have six or less white hairs on their foreheads. Regression toward more white has been as difficult to overcome as regression toward less white in the maximum line. This is true also of a new early stage plus line derived from the random bred line. A finely graded condition evidently exists in these animals, which with sufficient patience can be shifted from one grade to another. The fine gradations, and the amount by which the upper limit of variation in the plus line exceeds that of the random mated raises this question: What is the nature of the changes in the chromatin responsible for the breeding of much from little? Discussion desired. C4.

GOODGAL, S. H., The Johns Hopkins University, Baltimore, Md. Failure to obtain infra-red effects upon X-ray inactivated E. coli. — A 48 hour slant culture of E. coli B. was suspended in saline buffer to a

concentration of approximately 10^8/ml. and subsequently irradiated with X-radiation. The suspension was given a posttreatment with infra-red for periods up to 24 hours using a 250 watt drying lamp. In three series of experiments, the inactivation of E. coli by X-rays with a survival of 10^{-2} to 10^{-4} was not influenced by infra-red posttreatment. Temperatures were maintained at 0°C. for periods up to 3 hours and at 10°C. for the longer intervals. Platings were made with both the agar-layer and spreading techniques to eliminate possible temperature neutralization of the infra-red effect. — The results may be evaluated in the light of the following possibilities: (1) the experimental conditions were too limited to test the effects of infra-red on X-ray inactivation; (2) posttreatment was ineffective but pretreatment may not be; (3) the organism has no mechanism for responding to infra-red posttreatment; and (4) X-ray chromosome breakage and cellular inactivation are discreet unrelated processes. T5.

GORDON, HUGH and MYRON GORDON, New York Zoological Society, American Museum of Natural History, New York 24, N. Y. The frequencies of five genes for macromelanophores (cells capable of producing tumors) in seven natural populations of the platyfish, Xiphophorus (Platypoecilus) maculatus. — Macromelanophores are large black pigment cells that form a series of 5 identifiable patterns in the members of 7 geographically isolated populations of the freshwater platyfish of Central America. Each macromelanophore pattern is, in turn, controlled genetically by one of a series of 5 dominant, incompletely sex-linked alleles. The genes are as follows: Sp, spotted, for an irregular pattern on the sides; Sr, striped, for macromelanophores arranged in rows; Sd, spotted-dorsal, for black markings in the dorsal fin; Sb, spotted-belly, for ventral blackening; and N, nigra for black bands of large pigment cells along the flanks. The wild populations were collected in the following rivers, from north to south: Rio Jamapa, Rio Papaloapan, and Rio Coatzacoalcos in the State of Veracruz, Rio Tonala and Rio Grijalva in the State of Tabasco, Rio Usumacinta in Guatemala and the Belize River in British Honduras. Analysis of the frequencies of the 5 macromelanophore genes in the 7 populations indicates that each population is genetically distinct. Additional genetic differences between the populations are revealed by interspecific and intraspecific hybridization, by the presence, absence and the strength of macromelanophore gene modifiers—some of which suppress, others enhance the expressivity of the pigment cell genes. The modifier genes of pigment cell growth may be determined by mating a spotted member of one population with an unspotted member of a different natural population. The significance of these data to atypical pigment cell growth lies in the fact that macromelanophores are capable of initiating the development of melanomas in hybrids. Yet the growth of macromelanophores in all members of wild populations is normal. D2.

GOWEN, JOHN W. and JANICE STADLER, Iowa State College, Ames, Iowa. Viability of Drosophila melanogaster exposed to X-ray

irradiation. — 2180 drosophila adults have been irradiated once or more times with X-rays from a Coolidge type tube operated at 98 kvp and 2ma with filtration only that intrinsic in the tube. Treatment doses range up to 62,500 r. The flies were distributed in 5 factorial experiments. Twenty-six different criteria describe the physiological effects. Consideration is given to those factors which measure irradiation damage to the fly's life span and to the life of the progeny. Total eggs laid throughout the female's life presents a very complete measure of the functioning of the whole organism. Between 0 to 2500 r there was a 40 percent random variation in eggs laid. This seeming threshold indicates the female's physiological functions are not seriously altered. Irradiation fluxes of 2500 r or more show a linear decline in eggs laid as dosage increases; at 20,000 r productivity was but 2 percent, at 62,000 r less than 0.1 percent. Total days the female laid eggs measures the resistance to complete interference with functioning of reproductive system as distinct from rates of cell division. A threshold of little or no effect appeared between 0 and 2500 r. Days of egg laying were reduced linearly from 84 to 50 percent at 10,000 r. The capacity to lay eggs was reduced less than the eggs metabolized and laid. Life span of the females showed no reduction with exposures up to 12,500 r. Flies receiving 62,500 r lived about 60 percent as long as those treated with no treatment. Fertility in the males, percent of life males remained fertile and life span paralleled the like observations in the female. These observations show that quantitatively the sexes behave in similar manner when exposed to irradiation. E8.

GRANT, VERNE, Rancho Santa Ana Botanic Garden, Claremont, Calif. The significance of segregation in a raw allotetraploid. — Various hypotheses have been advanced concerning the type of genic system involved in the differentiation of intersterile species (systemic mutations, interspecific genes, polygenes, etc.). The presence of hybrid sterility makes it difficult or impossible to test these hypotheses by ordinary methods of genetic analysis. The problem can, however, be attacked by study of a raw allotetraploid derived by non-reduction from a sterile interspecific hybrid with some chromosome pairing. For such a hybrid will segregate for any interspecific differences determined by genes borne on the pairing chromosomes. Favorable material for this type of analysis is provided by two well-isolated diploid species of Gilia (Polemoniaceae). These species (millefoliata and achilleaefolia) differ in numerous morphological and physiological characters. Hybrids between them are usually difficult to produce and are invariably sterile with much reduction in chromosome pairing. Two different cultures of F_1s grown in two different years nevertheless spontaneously gave rise to a few vigorous and fertile allotetraploids, as a result of the union of unreduced gametes. The tetraploid F_2 segregates for certain parental characters (viz. herbage color, density of glands on stem, size of leaf segments, corolla size, relative size of corolla throat, length of stamens, etc.) which are determined, at least in part, by genes located on the few chromosomes which pair in F_1. Large F_3 and F_4 families

derived from single plant selections continue to segregate for these characters. (1) The F_1 is intermediate. (2) The variation in later generations is continuous and transgressive. (3) The segregating characters are weakly linked with more than 50% recombination. These facts suggest that the character differences which can be analyzed in this interspecific cross are individually controlled by multiple factors. B13.

GREEN, E. L., Ohio State University, Columbus, Ohio. Quantitative genetics of variation in number of presacral vertebrae in mice. — The P/JaxGn strain of mice has 25 presacral vertebrae (93%), 26 psv (3%), other types (4%). The SEC/2Gn-d strain has 26 psv (13%), 27 psv (70%), other types (17%). Matings between these two strains have produced percentages of mice with 25, 26, and 27 psv in F_1, F_2, F_3, and in first, second, third backcross generations consistent with a hypothesis of multiple gene inheritance and several developmental thresholds. C11.

GRIFFEN, A. B. and MARY WARTERS, Roscoe B. Jackson Memorial Laboratory, Bar Harbor, Me., and Centenary College, Shreveport, La. The centromeres and obscure chromosome parts in Drosophila. — The location and identification of the centromeres has been accomplished through the use of several chromosome rearrangements and ring fragments in D. melanogaster. Like the telomeres, centromeres do not reduplicate during the interphase stage which is represented by salivary gland cells. Four centromeres are present in each pair of synapsed homologous chromosomes, one for each original chromatid. Because of their mutual synaptic attraction to form a cluster, the centromeres are concluded to be identical and non-specific. In melanogaster, virilis, hydei and buscki the centromere cluster, visible in most interphase somatic cells, is the classic karyosome of nuclear cytology; this doubtless holds true in other organisms, but the body must not be confused with chromatic nucleoli which are known to result from heavily condensed sex chromosomes. The process of centromere location has revealed the nature and extent of the left arm of the X chromosome, of which the large non-chromatic nucleolus is a part, as well as the structure of the heterochromatic chromocenter; the morphological heterochromatin, whose loose organization and stainable heavy interband fibers are the only identifying features, is confined to chromosomes X, II, and IIIL, being completely absent from IIIR and the entire IV. Map positions for the centromeres and for the left arm of the X chromosome are given on a new map which has been withheld until the completion of the centromere study. D3.

GRIFFING, BRUCE, Iowa State College, Ames, Iowa. Contribution of genotypic and environmental effects to the relationships existing among tomato yield components. — An analysis of tomato yield was made in terms of yield components. Yield was first broken down into number of fruits and fruit weight. Number of fruits was further parti-

tioned into number of clusters and number of fruits per cluster. The objective was to study the contribution of genotypic and environmental effects to the relationships existing among yield components. — The experimental material consisted of a set of homozygous parents and all possible F_1's grown under field conditions in a replicated experiment. Data for all variables were transformed to logarithms for analysis. Genotypic regression and correlation coefficients were estimated by use of components (statistical sense) of variances and covariances. Pooled sum of squares and cross products assignable to "between plant within genotype" variation were used to estimate environmental statistics. — A within plant competition for common limited substrate was evidenced from the high negative environmental correlation (r = -.784) between number of fruits per cluster and number of clusters. Variation in fruit size was found to be independent of variation in total number of fruits for plants of the same genotype (r = -.056). This is explicable with the consideration that most variation in fruit size is due to competition among fruits within a cluster. The five variables were found to be highly correlated genotypically. The interpretation is that the correlated responses among these variables, for the most part, are due to pleiotropic manifestations of one gene complex, and the primary function of this gene complex is to control the balance between the competitive abilities of the opposing growth forces tending to, (1) increase number of reproductive parts, and, (2) increase size of these parts. J3.

GROSS, SAMSON R., Zoology Dept., Columbia University, New York City, N. Y. A biochemical requirement for the reproduction of phage T2r1. — It has been noted that a specific growth factor is required for the reproduction of T2r1 after adsorption onto autotrophic E. coli strain K 12. Cells growing logarithmically in the chemically defined medium—M9, were centrifuged and resuspended in a phosphate buffer supplemented with sodium chloride, exhausted under aeration for three hours at 37°C and then centrifuged and resuspended in buffer. A washed suspension of T2r1 was added to the bacterial suspension at a multiplicity of 0.01 to 0.1 and adsorption permitted to proceed. The suspension was then diluted in buffer and added to the growth media so that a 0.1 c.c. sample would contain 10 to 50 infected bacteria and aerated for an hour before titration for phage yield. Aeration in M9 repeatedly yielded no increment in virus titre while in nutrient broth a yield of twenty per infected bacterium was obtained. Yeast extract is as efficient as broth in promoting growth. The growth factor is only in the water soluble fraction of yeast extract, is heat stable, and dialyzable. None of the amino acids, vitamins, purines or pyrimidines tested singly have any growth stimulating effect. K3.

HAAS, F. L., E. DUDGEON, F. E. CLAYTON and W. S. STONE, University of Texas, Austin, Texas. Frequency of chromosomal rearrangements as related to rate of irradiation, temperature and gases.

— The frequency of translocations induced in the sperm of adult D. virilis males, irradiated at a rate of 2000r/min., was used to measure radiation damage. Comparisons were made of the rates of translocations when X-radiation was applied at 0-3°C and at 24-26°C, using air, 95%N_2 + 5%O_2, CO+CO_2+O_2, CO+O_2, or CO_2+O_2 as the gases in several experiments. Other reports have indicated that a reduction in the amount of oxygen present during irradiation (at relatively slower rates of irradiation) reduces the radiation damage. Although this seems true at room temperatures, it does not occur at 0-3°C where reduction in oxygen has not lowered the translocation rate to a marked degree. There is a greater difference between the frequencies of translocations irradiated in air at the low and high temperatures than there is between the frequencies of translocations irradiated at low temperature in air and in 5% oxygen. In some mixtures including CO, the frequency of translocations was reduced rather than increased, contrary to the situation reported in Tradescantia. It is obvious that the temperature and rate of irradiation are very important factors in determining the relations between the gases present and the amount of radiation damage. A10.

HEIDENTHAL, GERTRUDE, Union College, Schenectady, New York. X-ray Induced Recessive Lethals in Habrobracon. — A method for detecting x-ray induced recessive lethals has been developed as follows; Virgin females which have been forced to store 1st meiotic metaphase eggs were irradiated and then outcrossed to haploid males. F_1 virgins which developed only from eggs rayed in 1st metaphase were then tested by allowing each to lay eggs. These were counted and later examined for hatchability. Control hatchability for comparable F_1s was well above 90%; for no female was it as low as 50%. In the experimental series, any F_1 which laid eggs 50% or fewer of which hatched, was tallied as bearing one or more recessive lethals. The eggs counted were haploid; therefore, a recessive lethal on any chromosome, which would act prior to hatching of larva, would give approximately a 1 : 1 ratio of dead eggs to live larvae; those bearing two independently assorting lethals a ratio of 3 dead eggs to 1 live larvae etc. The data thus far indicate a ratio for one or more lethals of approximately 5.1% for 500r and 14.2% for 1000r. Comparable data for recessive lethals induced in sperm give 5.0% and 10.7% for the same dosages respectively. The above method has also been shown to be applicable to the much more resistant prophase stage. D17.

HINTON, TAYLOR and MARGARET R. ROBERTS, University of California at Los Angeles, Los Angeles, Calif. Apparent Mendelian and non-Mendelian nucleic acid requiring "mutants" in Drosophila. — Raising Drosophila on a chemically defined medium under aseptic conditions, the nucleic acid requirements of a number of strains were studied. The wild-type strain tested, as well as several strains carrying visible mutants, did not require nucleic acid in the diet for growth

and development. On the other hand, several strains tested failed to develop in the absence of nucleic acid. Two of these nucleic acid requiring strains have been analyzed. Even though adenine was shown to satisfy the nucleic acid requirement in both strains, certain striking differences exist. When strain #1 is crossed, in various ways, to non-requiring strains, no offspring carrying a marked second chromosome from strain #1 appear in the absence of nucleic acid in the diet. The requirement acts as though it depended upon an orthodox second chromosome dominant gene. When exactly the same crosses are made using strain #2 instead of #1, all types of chromosome combinations appear in the offspring with the frequency expected were no nucleic acid requirement present. So far, attempts to recover the requirement by further chromosome recombination have failed. The strain behaves as though it has acquired, as a result of the outcrosses, some non-chromosomal factor that restores its ability to synthesize nucleic acid. However, when strain #2 is crossed to #1, no offspring appear with a marked second chromosome from either strain. In this cross both strains appear to have their nucleic acid requirement dependent upon a second chromosome dominant factor. The results can be interpreted if both chromosomal and non-chromosomal factors are assumed. G1.

HOROWITZ, N. H. and M. FLING, California Institute of Technology, Pasadena, Calif. Genetics of tyrosinase thermostability in Neurospora. — Investigations of tyrosinase in nutritionally wild-type strains of Neurospora have shown that strain differences exist with respect to thermostability of the tyrosinase activity obtained in crude and partially purified extracts. Extracts from some strains (TL) are half-inactivated in 4 minutes at 59°, whereas with other strains (TS) the half-inactivation time is 40 minutes or longer. At 35° the half-inactivation times are approximately 6.5 and 15 hours, respectively. Both enzymes are rapidly destroyed by boiling. The difference in thermostability has persisted through a 13- to 17-fold purification of the enzymes, based on protein nitrogen. This and other evidence suggests that the enzymes are structurally different. — Analysis of 40 asci from crosses of TS x TL indicates that thermostability and thermolability are inherited as if determined by alleles of a single gene. The locus is at least 29 map units from the centromere. Its linkage group is unknown. — TS and TL do not differ phenotypically under the conditions so far studied. In early experiments it appeared that TL determines a phenotype (H) characterized by normal production of tyrosinase at 25° and little or no production at 35° (analogous with Himalayan in mammals), while TS appeared to determine the phenotype S, in which tyrosinase is produced at either 25° or 35° (analogous with self-colored in mammals). Recent experiments indicate that this is not invariably true. Some H strains are TS, and some S strains are TL. T6.

HOUSE, VERL L., The Johns Hopkins University, Baltimore, Md. The interaction of some mutants affecting venation in Drosophila mela-

nogaster. — The interaction of three mutants (cubitus interruptus, ci; engrailed, en; Hairless, H) producing an interruption of the fourth longitudinal vein (L_4) has been investigated. Using as an index to phenotypic effect the average percentage of L_4 present relative to normality, it is found that the three mutants interact superadditively in the various combinations, although the data agree fairly well with an additive interpretation when subthreshold actions of the three mutants are taken into consideration. Heterozygotes of en and ci act as enhancers of ci and en homozygote actions respectively; such enhancement being interpreted on a basis of subthreshold components of action in the heterozygotes. Certain mutant combinations show venation effects other than L_4 interruption, these effects not being found in single mutants. With respect to mutant attributes other than L_4 interruption, mutant interaction may be inhibitory as well as cumulative. The frequency of venation has been determined at each of twenty intervals along the length of L_4 for the three mutants and their combinations. An inverse probability transformation is used to transform the frequency of venation at any L_4 interval into a quantity representing the difference between two concentrations, $\overline{P}$ and $\overline{P}_T$; $\overline{P}$ representing the mean value of a substance necessary for vein formation, $\overline{P}_T$ being the threshold value P must exceed in order to produce vein. Transformed frequency profiles of L_4 venation have been constructed for all genotypes. Analysis of these profiles reveals differential responses for different regions of L_4 to changes in genotype and environment. L8.

HUESTIS, R. R. and RUTH WILLOUGHBY, University of Oregon, Eugene. Inherited differences in a vertebral column. — A prominent feature of the vertebral column of Oregon Peromyscus maniculatus is a single, relatively very long spinous process. This long process, typically located on the second thoracic vertebra, may be absent in some individuals or, in a minimum number of cases, may be located on the third thoracic vertebra. These three types of individuals will be called P_2, P_0, and P_3 respectively. In a cage-bred population of 332 mice, investigated to establish a norm, 80 percent were P_2; 13 percent were P_0; and 7 percent were P_3. — Selection of P_0 individuals for breeding, which may be done by feeling between the scapulae, significantly increases the incidence of both P_0 and P_3 mice in succeeding generations and at the same time significantly increases the average number of lumbar vertebrae and the relative number of individuals which have 14 rather than 13 rib pairs. It is tentatively assumed that genes operate by locating the stimulus for the development a vertebra of a given type further caudad in the developing vertebral column. The lack of a spinous process, in P_0 mice, may be due to the location of the stimulus to form a single long spinous process opposite cells which are not competent to form one. T7.

JAMES, A. P., Atomic Energy of Canada, Ltd., Chalk River, Ontario. An exceptionally frequent induced genetic change in yeast. — A

galactose positive strain of S. cerevisiae mutates readily under the influence of 2537 Å ultraviolet to galactose negative. The change differs from most induced gene mutations in that: (1) it occurs at a very high rate, affecting as many as 10 percent of the colonies from irradiated cells; (2) it is detectable in the diploid without breeding for homozygosity; (3) the ratio of whole-colony to sectored mutants increases with increasing dose; (4) the instability is perpetuated over many generations in certain galactose positive lines from irradiated cells; (5) the likelihood of the induced change varies widely in lines obtained on selfing the original line, and this variability can be observed even after eight generations of inbreeding. The data are so far not incompatible with the concept of mutation by loss (or partial loss) of a particular type of cytoplasmic particle. G10.

KALTER, HAROLD and F. CLARKE FRASER, McGill University, Montreal, Quebec, Canada. Hereditary differences in the reaction of mice to the teratogenic effects of cortisone. — By treating pregnant female mice with cortisone, gross congenital deformities can be induced in the fetuses. By far the most frequently occurring of these is cleft palate (CP). Cleft palate, without cleft lip, has not been observed in the offspring of untreated females of the strains used. — The frequency of CP varies with 1) the strain of mice, 2) the dose of cortisone, and 3) the day of gestation on which treatment is begun. Figures below are percent CP resulting from treatment begun on day 10 of gestation and total offspring in each case. Strain A/Jax is highly susceptible (100%, 32); strain C57BL/6 Jax, relatively resistant (19.8%, 81). The cross C57BL ♀ x A ♂ gives 4.3% CP (69), showing that the hybrids are more resistant than either parent strain. The reciprocal cross A ♀ x C57BL ♂ gives 51.6% CP (31), showing that in addition to heterosis there is an effect of the maternal constitution. Two additional crosses have been made to determine whether or not the maternal effect is cytoplasmic in nature. The cross (A ♀ x C57BL ♂) ♀ x A ♂ gives 18.2% CP (22), and (C57BL ♀ x A ♂) ♀ x A ♂ 29.2% CP (24). These are not significantly different. — It seems, therefore, that a genetically influenced maternal-fetal relationship contributes in some manner to the production of cleft palate by cortisone. D4.

KAY, ROBERT and BERNARD PHINNEY, University of California at Los Angeles, Los Angeles, Calif. The control of chlorophyll and carotenoid production by a virescent gene in maize. — The virescent, pale yellow seedling, is a single gene mutant controlling both carotenoid and chlorophyll accumulation. Light-grown mutants are pale-yellow; dark-grown mutants are also pale-yellow while dark-grown normals are bright-yellow. At room temperature the pale-yellow character persists for about two weeks. Thereafter mutants begin to green and one week later are as green as normals. Previous light exposure, variations in culture media, and removal of endosperm does not influence the length of time that mutants are pale-yellow. Plastid size and num-

ber are not a factor. However, the length of time that mutants remain pale-yellow is inversely proportional to temperature. This lag in time for initial greening is attributed to a lack of a "threshhold" amount of an enzyme, the accumulation of which is temperature dependent. In addition the temperature range over which greening will occur is narrower indicating an altered enzyme in the mutant. — Dark-grown mutants are low in carotenols and protochlorophyll, but not in carotenes. Light-grown mutants are deficient in carotenols, chlorophylls and carotenes. Seedlings grown without endosperm show that mutant seedlings are probably unable to form carotenols. Accumulation of carotenols and chlorophylls(or protochlorophyll) begins at the same time in either dark-grown or light-grown mutants. This accumulation coincides with the visual appearance of greening. Thus the mutant may control the synthesis of carotenols and chlorophylls through the interruption of the synthesis of a common precursor. Carotene synthesis in light-grown mutants is interrupted indirectly probably through the lack of a product of photosynthesis necessary for carotene formation. M3.

KIMBALL, E., Clinton Experimental Farm, Clinton, Conn. Genetics of primary plumage patterns in the fowl. — A primary plumage pattern gene is defined as a factor determining pterylar and multipterylar distribution of a specific black feather pigment. All known primary patterns in the fowl are attributable to action of multiple alleles at the E locus. The commonly-encountered alleles are e^+ (wild type), e (columbian), and E (extended black). Breeding experiments demonstrate that each primary pattern gene is pleiotropic in action. Principal aspects of this pleiotropy are (1) a pigmentation phase, and (2) a restriction phase. Biochemical identification of black feather pigments is as yet inadequate, but well-marked genetic and physiological differences are demonstrable. The pigments e^+ black and e black are closely related, and sharply differentiated from E black. Dominance relations of pigmentation phases are expressed in the order $E > e > e^+$. As regards restriction phases, gene e^+ is characterized by wild type pyle-zoning, gene e by columbian belt-zoning, and gene E by complete absence of restrictor potential, i.e., e^+ is normal, e is a hypermorph, and E is an amorph. Phenotypic complexities of heterozygotes, viz., ($E\ e^+$), ($E\ e$), and ($e\ e^+$), derive from interaction of pigmentation and restriction phases. Analysis of data provided by critical breeding experiments reveals that phenotypes are predictable on the basis of pigmentation and restriction potentials predicated above. Variance in gene potency incident to maturation, and influence of estrogens on feather germs must be given due weight in accounting for sexual differences in down, juvenile, and adult phenotypes in heterozygotes. T8.

KIMBALL, R. F. and NENITA GAITHER, Oak Ridge National Laboratory, Oak Ridge, Tenn. The role of externally produced hydrogen peroxide in the action of X rays upon Paramecium aurelia. — Nongenetic death and division delay in Paramecium are produced by 50-

400 kr of X rays depending on the medium and other conditions. Irradiation in a medium in which accumulation of H_2O_2 was prevented by living bacteria or by catalase was less effective than irradiation in a medium containing small amounts of organic matter but no living bacteria or catalase. Irradiated medium of the latter kind produced death and division delay in paramecia added after irradiation. The doses required were only slightly, if at all, greater than those which, given directly to the animals, produced an equivalent biological effect. The activity of irradiated medium was destroyed by catalase and tests for H_2O_2 were positive. The amount of H_2O_2 found was two-thirds to three-quarters that which, added directly, produced an equivalent biological effect. Thus a large part of the action of irradiated medium can be attributed to H_2O_2 although some must be brought about by other mechanisms. — The medium in which the paramecia were irradiated had no influence on the amount of mutation nor were mutations induced in detectable amounts by sublethal exposures to H_2O_2 or irradiated medium. Thus the large number of mutations induced by direct irradiation with doses of this magnitude must have been due to energy absorbed in the animals. It is not clear whether substances produced in the medium are not mutagenic or cannot penetrate to the micronuclei. A11.

KING, R. C., Brookhaven National Laboratory, Upton, L. I., New York. The Mutagenic Effectiveness of Radioactive Phosphorus in Drosophila melanogaster.* — The mutagenic effectiveness of radiophosphorus in D. melanogaster has been studied using the Muller-5 method for recovering sex-linked recessive lethal mutations. Methods are described for varying the radiation dose from the radioactive medium and the dose from P^{32} within tissue with respect to each other. The radiation dose from the medium is determined by actual measurement, while the radiation dose to the male gonad from P^{32} in tissue is calculated. It is shown that radiations from internally located P^{32} atoms are mutagenically effective. However, in the case of male Drosophila fed upon P^{32} as adults there is no indication that internal P^{32} is more efficient mutagenically than external P^{32}. Among the genetic changes recovered following P^{32} treatment are sex-linked recessive lethal and viable mutations, inversions and dominant lethals. P^{32} treatment also increases somatic crossing over and produces potential chromosome breaks leading to the production of unilateral mosaicism. The importance of this work in the study of genetic effects ascribable to the actual transmutation of P^{32} to S^{32} is discussed. E4.

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*Part of the research was carried out at Brookhaven National Laboratory under the auspices of the Atomic Energy Commission, while the rest represents a portion of a dissertation, presented for the Ph.D. degree in Yale University during tenure of a Public Health Service Fellowship.

KIRBY-SMITH, JOHN S. and D. S. DANIELS, Biology Division, Oak Ridge National Laboratory, Oak Ridge, Tenn. The relative effects of X rays, γ rays and β rays on Tradescantia pollen. — Tradescantia pollen grains have been irradiated with 250 kvp X rays filtered by aluminum and copper, γ rays from Co^{60}, and β rays from P^{32}. Following irradiation the material was cultured on a lactose-agar medium with colchicine and chromosomal aberrations analyzed at the pollen tube division. Complete dose-aberration frequency curves based on large numbers of cells have been obtained for the different radiations in a number of independent experiments. In every instance the X rays were found to be approximately twice as effective in producing chromosomal aberrations as the same dose expressed in equivalent roentgens of high energy γ rays or β rays. These results indicate that the coefficients of chromosomal aberration production in Tradescantia are not constant over the wave-length range from medium-energy X rays to high-energy γ rays, as has been previously believed. At all wave lengths studied the frequency of isochromatid breaks has been found to increase with dosage more rapidly than the generally assumed linear relation. This last finding confirms results previously reported by Catcheside, Lea, and Thoday. E3.

KITZMILLER, J. B. and C. C. CLARK, University of Illinois, Urbana, Ill. Salivary gland chromosomes in Culex mosquitoes. — Maps of salivary gland chromosomes of Culex pipiens and Culex quinquefasciatus are being constructed. The chromosomes are best from fourth instar larvae which have been reared in uncrowded conditions and with ample yeast food. Each gland is about 0.7 mm long, with a proximal tubular portion about 150 microns wide, consisting of about 30 cells, and a spherical distal portion about 200 microns in diameter, composed of about 30 slightly larger cells. Only a few of the nuclei of the proximal portion have yielded chromosomes suitable for study. The glands are dissected in 0.7% NaCl solution, transferred to acetocarmine for at least four minutes, then are squashed on a slide with a cover slip. Suitable chromosomes are about 3 microns in diameter, but with varying diameters along their lengths. They have distinct bands of varying intensities. Chromosomes of younger or of poorly fed larvae are smaller and have less distinct banding. Many cases have been seen of chromosomes dividing in a small region into parallel branches, each of half the initial diameter and with non-homologous banding in the two branches, which subsequently rejoin. In many other regions there is a partial splitting of the chromosomes, but with homologous banding in the branches. These observations indicate synapses of homologues. Three salivary gland chromosomes are seen suggesting a diploid number of six, which is in agreement with known chromosome numbers of the genus. There is no obvious chromocenter. H6.

KONZAK, C. F. and W. R. SINGLETON, Brookhaven National Laboratory, Upton, L. I., New York. The Relationship of Polyploidy to the

Effects of Thermal Neutron Exposure on Plants.* — Dry seeds of diploid (2N), autotetraploid (4N) rye, barley and maize; 2N, 4N and 6N wheat and oats were exposed to neutrons in the thermal column of the nuclear reactor for periods of 4, 8, 12 and 16 hours. The flux was approximately 4.6 x 10^8 $N_{th}/cm^2/sec$. The cadmium ratio was about 10,000 : 1 and gamma contamination was about 100 r/hr. In addition, some comparisons were undertaken using X-rays (250 Kv, 30 ma. 0.1 mm Al, hvl 0.2 mm Cu, 1600 r/min). — Autotetraploid rye, barley and maize were less injured following thermal neutron exposure than were the diploids as measured by seedling growth. Tetraploid oats were distinctly more resistant to injury caused from thermal neutron bombardment than was the hexaploid or the diploid. The same material responded to X-radiation in the expected manner, i.e., the species having the highest chromosome number was the most resistant. The results with wheat were similar to those with oats after thermal neutron exposure although somewhat less extreme. — The uniformity of the injury of seedlings grown from seeds exposed to thermal neutrons was especially noteworthy in view of the variable effects frequently obtained with the same material exposed to X-rays. These results indicate that the effects of thermal neutrons and X-rays are not parallel. Cytological analyses of root-tips from seeds exposed to thermal neutrons are now in progress. E10.

LARSON, RUBY I., Field Crop Insect Laboratory, Lethbridge, Alberta, Canada. Aneuploid analysis of inheritance of solid stem in common wheat.** — Two varieties of common wheat, Chinese Spring (essentially hollow) and S-615 (essentially solid), differ by four major genes affecting stem solidness. Aneuploid analysis showed these genes to be a solidness promoter on chromosome VIII and solidness inhibitors on chromosomes XIII, XIX, and XX of Chinese Spring. In both parents, chromosome II is weakly and XXI strongly inhibitory. Chromosomes I, IX, X, XI, and XII appear to be needed in disomic condition to produce full stem solidness. On the basis of effect on stem solidness, chromosomes I, VIII, IX, X, XI, XII, and XIII are believed to constitute the B genome, and II, III, IV, V, VI, VII, and XIV (not tested) the A genome. Each such postulated genome contains one member only of each of the seven homoeologous series of three chromosomes that can compensate physiologically for one another and that presumably belong to genomes A, B, and D respectively. Four of the genes affecting stem solidness discovered by Yamashita (Mem. Coll. Agric., Kyoto Imp. Univ. 39: 9-38, 1937) in interspecific wheat hybrids have been located as to chromosome. The weak pith inhibitor, m_A, in the A genome is probably on

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chromosome II. The gene for solidness, M_B, in the B genome may be the null allele of m_B, a solidness suppressor on chromosome XIII. The epistatic pith inhibitor, O_D, is likely on chromosome XX. Hollow-stemmed varieties of common wheat probably possess pith inhibitors on chromosomes XIII, XIX, and XX that S-615 does not have. J5.

LAUGHNAN, J. R., University of Illinois, Urbana, Ill. The A^b components as members of a duplication in maize. — The anomaly of close linkage between genes with similar phenotypic effects is explained without assuming duplication if it is argued, with Goldschmidt, that the mutant forms represent modifications at different points within a chromosome segment the whole of which is concerned normally with development of a specific phenotype. Alternatively, the close linkage between such genes may trace to their origin from an ancestral locus via duplication in which case the problem has significance from an evolutionary standpoint. Genetic studies indicate that the components of A^b (designated α and β) are members of a duplication: (1) From A^b/a plants both stable and mutable A^d (α component) derivatives occur via crossing over. Likewise from A^b/a^b individuals A^d derivatives representing different levels of effect are obtained in association with crossing over. In both instances the actions of the derivatives suggest that they originate from crossover events following variable synaptic patterns in sporocytes of the parent individuals which in turn leads to the inference that α and β are homologous or lie in homologous segments. (2) The yield of A^d derivatives from A^b/a plants heterozygous for the translocation 2-3d is greatly reduced in T A^b/a individuals but is unchanged, if not enhanced, in T a/A^b plants. This effect on crossing over between α and β does not hold for segments adjacent to A^b. (3) A critical test of the duplication hypothesis is provided by the A^b/A^b compound which, on the basis of homology between components, is expected to yield A^d following crossing over between α and β in obliquely synapsed strands. Such derivatives occur and their frequency is too high to be explained solely by point mutation of A^b. Moreover, A^d rates are drastically reduced in T A^b/A^b plants, a result which is not expected if point mutation is the sole basis for the origin of A^d in the homozygote. M4.

LEFEVRE, G., JR. and G. D. HANKS, University of Utah, Salt Lake City, Utah. The frequency of X-ray induced white and Notch mutations in an apricot Abruptex stock as compared with wild-type in Drosophila melanogaster. — The incidence of white and Notch mutations was determined following exposure of apricot (w^a) Abruptex (Ax) and Canton-S + males to an X-ray dose of 5000r. Ax contains a duplication of salivary chromosome band 3C7, deficiency of which produces Notch. Ax/N heterozygotes are phenotypically non-Notch. From w^a, 30 white changes were detected in about 25,000 F_1 females inspected; from +, 24 whites in 24,000. In each case approximately 1/2 of the whites were male-lethal (deficiencies?) and 1/2 were male-viable. From Ax, 20

Notch mutants were found in 22,000 F_1 females; from +, 24 Notches in 24,000. Thus, no significant effect was noted of irradiating w^a rather than w^+, nor of exposing Ax rather than +. — Since approximately 1/3 of all Notches are cytologically normal or have only one band missing following + irradiations (data from Bridges and Brehme, 1944), if the extra band in Ax is a functional duplication of 3C7, then fewer Notches would be expected from Ax than from +. Since the incidence of Notches is actually the same, apparently the extra band in Ax is incapable of covering the loss of the neighboring 3C7 band, because of a position effect, even though the extra band normally suppresses the Notch phenotype resulting from the loss of band 3C7 from the homologous X-chromosome. — The similar frequency of whites in the w^a and + irradiations conflicts with data of Timofeeff-Ressovsky (1932), but supports the concept of the destructiveness of X-rays in Drosophila. E6.

LEVINE, MYRON, Indiana University, Bloomington. Diverse mate-killers: their interrelations and genetic basis. — Three stocks (130, 131, and 138) of variety 8, Paramecium aurelia, have been examined for hereditary differences in the mate-killer trait. This trait is known to be controlled by the cooperative action of a nuclear gene and visible cytoplasmic particles. (See abstract by R. W. Siegel in these records). Sensitives mated to slowly reproducing animals of these stocks regularly die before completing two fissions. The exconjugants cytoplasmically descended from stocks 130, 131 and 138 always produce viable cultures. Sensitives mated to rapidly grown mate-killers of stock 138 produce a limited number of daughter cells, all of which are doomed to die. However, sensitives mated to rapidly grown mate-killers of stocks 130 and 131 are only temporarily retarded in their early reproduction, then recover and produce normal viable cultures. When crossed to one another, the mate-killers exhibit a system of striking interactions. Each cross exhibits the typical mate-killer reaction: one conjugant regularly dies and the other survives. This system of interactions gives rise to a "peck-order" of mate-killing which according to strength of mate-killing is: 138—130—131. An analysis indicates that the seat of specificity of the differences in mate-killing between the mate-killer stocks resides in the cytoplasmic particles rather than in the nuclear genes. K8.

LEVINE, R. P. and J. I. DICKINSON, Amherst College, Amherst, Mass. The Modification of Recombination by Naturally Occurring Inversions in Drosophila pseudoobscura. — Carriers of third chromosome inversion heterozygotes gave more X-chromosome recombination in regions y-sn and sn-v than did carriers of homozygous arrangements. The gene arrangements studied were Standard (ST), Arrowhead (AR), and Chiricahua (CH), for each combination of which approximately 1,500 flies were classed. The order of the amount of recombination found was: ST/CH>ST/AR>AR/CH>ST/ST = AR/AR = CH/Ch, the dif-

ferences indicated having P values of less than 0.05. The observed values ranged from 18.2% for ST/CH to 11.0% for CH/CH. — Carriers of third chromosome inversion heterozygotes have been shown to have an adaptive value superior to that of inversion homozygotes in natural populations and in population cages, presumably through the maintenance of superior gene combinations by the suppression of crossingover in the inverted segments. Since carriers of these inversion heterozygotes show an increase in crossingover in another chromosome, an increased production of new genotypes is assured. Thus by a single mechanism, inversion heterozygosis, a population maintains both its adaptedness and its adaptability. L6.

LEVITAN, M., Virginia Polytechnic Institute, Blacksburg, Va. A ring chromosome in wild-caught Drosophila. — An inseminated Drosophila robusta female collected in June, 1951 near Blacksburg, Virginia, produced some offspring whose salivary chromosomes showed fusion of the two distal ends of the small, nearly metacentric Chromosome 3. The right arm involved carried the common gene arrangement 3R-1, which differs from arrangement 3R by a subterminal inversion of most of this arm. Upon desperming and crossing the female with males of known genotype, she produced eggs carrying 3R, 3R-1 in an open chromosome, or 3R-1 in the closed chromosome. The data indicate the new aberration developed in her germ line. That the aberration is a true ring has been confirmed by neuroblast smears. The salivaries show no detectable deficiencies. Males and females from this stock that are heterozygous for the ring show good viability and fertility. A late third instar larva was found homozygous for the ring; whether adults homozygous for it exist has not been determined. The ring was seen again in one larva from a female collected in July, 1951. However, the second female did not carry the ring; nor has it been found again in the stock derived from here. This is probably the first recorded instance of a ring chromosome from a natural population of animals, and it may be the only instance thus far of a ring involving a complete chromosome. Further study will indicate whether it has persisted in the wild population. L2.

LEWIS, E. B. and W. GENCARELLA, California Institute of Technology, Pasadena, Cal. Claret and non-disjunction in Drosophila melanogaster. — A new X-ray induced mutant, claret-non-disjunctional (ca^{nd}), in D. melanogaster has the typical claret eye-color but is associated with high egg mortality and peculiar effects on chromosome disjunction previously known only for the claret mutant of D. simulans (Sturtevant, Wald). Among 1373 progeny of females homozygous for ca^{nd} which had been mated to males carrying the sex-linked mutants, yellow and Hairy-wing, 42.9% were regular offspring; 15.0% were exceptional (XXY or XO): 32.0% were wholly haplo-IV (of these 404 were regular, 35 exceptional); 3.9% were haplo-IV mosaics (of these 49 were regular, 5 exceptional); 5.4% were gynandromorphs (among these all of

the above types were represented); and the remaining 0.8% included a triploid female, triploid intersexes, probable superfemales and superfemale-female mosaics, a regular-exceptional mosaic male (XY-XO), a similar type of mosaic female (XX-XXY), and finally a mixed XXY-XX-XY fly. The three latter mosaic flies probably resulted from double fertilizations, for the effect of ca^{nd} is otherwise restricted to the maternal chromosomes, as with claret of simulans. The ca^{nd} mutant provides another tool for the study of chromosome mechanics and for the study of gene action in mosaics. T9.

LIEB, MARGARET, California Institute of Technology, Pasadena, Calif. The production of lysogenic strains of bacteria. — When cells of a sensitive (non-lysogenic) strain are exposed to temperate phages, only part of them lyse after a brief latent period. Among the surviving cells are some which give rise to colonies containing lysogenic cells, which carry the phage in a latent form and transmit the lysogenic trait to all their offspring. The study of individual clones derived from single lambda-infected E. coli K12S cells revealed that cells which give rise to lambda-carrying cells do not, in addition, give rise to cells that lyse. The formation of carried phage, resulting in lysogenicity, and the multiplication of phage leading to lysis apparently are mutually exclusive processes in an infected cell. The decision as to which process is to prevail is made by the bacterium either before or immediately after infection. However, during the first hour after infection, the establishment of lambda phage can be suppressed in some or all of the cells of a clone by growing the cultures at temperatures above 20°C. Such temperature treatments have no effect on cells of established lysogenic cultures; there is, in fact, no method known for systematically transforming lysogenic K12 strains into sensitive strains. These findings suggest that the infecting phage which does not lead to lysis acts as a cytoplasmic particle immediately after infection, and later becomes attached to a permanent cell constituent, so that lysogenicity becomes a hereditary trait. This interpretation will be discussed in relation to the similarity of the behavior of lambda to that of kappa in Paramecium, and to the available genetic findings. K1.

LINDEGREN, CARL C. and GERTRUDE LINDEGREN, Southern Illinois University, Carbondale, Ill. The Tripartite Nature of the MZ Gene System in Saccharomyces. — The MZ gene exists in Saccharomyces as a series of multiple alleles which differ in competence with regard to the fermentation of sucrose, alphamethyl glucoside and melezitose. A culture carrying the fully competent allele, MZ, can adapt to maltose, turanose, sucrose, melezitose and alphamethyl glucoside. MZ^a cannot adapt to alpha-methyl glucoside; MZ^b cannot adapt to melezitose; MZ^c cannot adapt to either melezitose or alpha-methyl glucoside, and MZ^d can respond adaptively only to maltose and turanose. All alleles respond on adaptation by producing the same enzyme: An MZ^d culture which can grow in only maltose or turanose and not in

melezitose, alpha-methyl glucoside nor sucrose, after adaptation to maltose, can ferment all five sugars. The gene-system acts specifically in the elicitation of enzyme, but non specifically in the production of enzyme. It is inferred that the MZ gene-system has three parts: (1) a highly specific receptor controlling the reaction between the gene and the eliciting substrate, (2) a non specific effector controlling the production of enzyme and (3) a means of communication between these two different sites. G8.

LINDSLEY, D. L., Princeton University, Princeton, New Jersey. Spermatogonial exchange involving In(1)sc^{8}.* — Among the progeny of males carrying In(1)sc^{8}, individuals are found which have lost the terminal euchromatic segment of the inversion. This has been interpreted (Sidorov 1940, 1941) as the result of exchange between the distal heterochromatin of the inversion and the short arm of the Y chromosome, and, indeed, the reciprocal recombinant type may be recovered, i.e. a Y with its short arm replaced by the terminal euchromatin of In(1)sc^{8}. — An analysis of 44 such recombinant X chromosomes recovered from 273,721 offspring of males carrying the distal heterochromatin of In(1)sc^{8} reveals that previous notions represent an oversimplification of the actual case. Among the recombinants studied the Y chromosome seems to have been a relatively rare participant, at least in those crosses in which the paternal chromosomes carried large proximal heterochromatic segments. Analyses of recombinants indicate that the distal heterochromatin of In(1)sc^{8} may pair with any other sex-linked heterochromatic segment with the possible exception of Y^{L}; such pairing may occur in either direction as if there were no linear differentiation of pairing specificities in heterochromatic segments, or as if a reverse repeat system were operating. Inversion of ordinary pairing relationships followed by exchange may result in the formation of dicentric bridges; the evidence indicates that such bridges break at anaphase. — No evidence has been found favoring any sort of fusion cycle subsequent to bridge breakage, but the experimental arrangement favored detection of only a chromatid type of cycle. D5.

LOVELACE, ROBERTA, Oak Ridge National Laboratory, Oak Ridge, Tenn. and University of South Carolina, Columbia. (Introduced by Mary Esther Gaulden). The effects of pre- and posttreatment with ultraviolet radiation on the frequency of gamma-ray-induced aberrations in Tradescantia chromosomes. — Growing pollen tubes were irradiated with ultraviolet radiation of wave lengths longer than 2600 A isolated from an intermediate pressure mercury arc (Hanovia SC 1550) by means of Corning filter #9700. The transmission of this filter rises rapidly from a cutoff at 2600 A to approximately 90% at 3200 A and it

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*Results of research carried out at the California Institute of Technology under a National Research Council administered AEC Predoctoral Fellowship.

is essentially transparent throughout the near-ultraviolet and visible range. The cells were given 250 r of radiation from a Co^{60} source one hour after about 0.3×10^6 ergs/sq cm of ultraviolet, in the wave-length range 2600-3600 A, was delivered to them. Results of these experiments indicate that gamma-ray-induced isochromatid aberrations are increased and exchanges are decreased by pretreatment with ultraviolet radiation. — Further experiments using radiation from an AH 4 lamp transmitted by a number of short wave-length cutoff filters and with higher doses of ultraviolet radiation have been carried out. In addition, essentially monochromatic radiation of wave length 3650 A has been used. Preliminary data on these experiments indicate that the modification of gamma-ray-induced aberrations by ultraviolet radiation is dependent upon wave length, dosage, and the interval of time between the two treatments. E2.

MAKINO, S., Hokkaido University, Sapporo, Japan and Colorado Agr. College, Fort Collins, Colo. Presence of the strain cells in ascites sarcomas of rats and their chromosome constitutions. — In the Yoshida sarcoma, an ascites tumor of rats, a strain of tumor cells is present, which have their own characteristic chromosome constitution and multiply by regular mitosis. They possess well-balanced ± 40 chromosomes consisting of 22 to 24 rod-shaped ones which probably come directly from the original tissue cell, and 16 to 18 V- and J-shaped chromosomes which are specific for the tumor cells. Their origin is unknown, probably mutational in character. Because of this peculiarity, the chromosomes of tumor cells are markedly different from those of the host cells which are characterized by 42 rod elements. The individuality of the chromosomes in the strain cells remain unchanged during successive transplant generations. The growth of the tumor is primarily caused by the proliferation of these strain cells. In the course of multiplication part of the proliferating cells become abnormal owing probably to the spindle disturbance, structural changes of chromosomes, and some other causes. Frequently occurring tumor cells showing abnormalities are derivatives of the strain cells. Destruction of derivative cells by chemical treatment is followed by multiplication of the resistant strain cells. Comparative evidence has been found in two new strains of ascites tumors similar to Yoshida sarcoma. Their strain cells have a similar chromosome number, and within the set, the same two groups of rod- and of V- or J-shaped chromosomes, but differ from each other as well as from Yoshida sarcoma in the number of V- and J-shaped chromosomes. J1.

MARKERT, C. L., University of Michigan, Ann Arbor, Michigan. Genetic control of glutathione synthesis in Glomerella. — A peptide-requiring mutant (502-1) of the fungus Glomerella has been induced by X-radiation. From this original mutant a second peptide mutant (502-2) was obtained as a spontaneous sector. These two peptide mutants will grow on media supplemented with the tripeptide glutathione (glutamyl-

cysteinylglycine) but not on any mixture of the constituent amino acids, thus suggesting that the formation of a peptide bond has been affected by mutation. The two mutants, 502-1 and 502-2, may be distinguished by their appearance on complete medium as well as by their growth responses to the two dipeptides into which glutathione may be resolved. Both mutants grow only slightly on cysteinylglycine but show good growth on a mixture of cysteinylglycine plus glutamylcysteine. Mutant 502-1 will grow on glutamylcysteine alone, but 502-2 does not grow on this dipeptide. — Genetic analysis of these mutants reveals that 502-2 segregates from a cross of 502-1 to wild type, suggesting that 502-1 differs from wild type at two loci: one controlling glutathione synthesis and the other controlling growth on glutamylcysteine. However, segregation ratios are aberrant and two unusual types of segregants are observed: (1) segregants which die after slight growth and (2) segregants with drastically altered morphology. It seems probable, therefore, that a chromosomal aberration is involved in the formation of these peptide mutants. G9.

MEYER, HELEN U. and H. J. MULLER, Indiana University, Bloomington, Indiana. Influence of oxygen and of temperature on the rate of autosomal recessive lethals induced by ultraviolet in the polar cap of Drosophila melanogaster.* — Two lots of embryos shaded except for their polar caps were treated simultaneously with uv. from a Germicidal lamp, with "photoreactivating" wave lengths removed, in atmospheres of oxygen and nitrogen, respectively. Both lots were again divided into two groups, placed 5 minutes after treatment at 17°C and 27°C, respectively, and thereafter cultured at these temperatures. In the 5-minute interval the embryos in polar cap stage were chosen. In the main (large-scale) series of the work, the exposure lasted 50 to 60 seconds, delivering the intentionally low dose of 90 to 108 ergs/mm^2. The gases were administered from 5 minutes preceding until 2 minutes following irradiation. — Sons of males whose pole cells had been irradiated were scored for recessive lethals in their second chromosomes, by a special genetic technique utilizing sterility genes. With both temperatures, higher mutation rates were obtained from nitrogen treatment than from oxygen. In both atmospheres, lots subjected to 17°C gave higher mutation rates than those given 27°C. The percents of lethal mutations in the main series, based upon 700 to 800 tested chromosomes in each of the 4 groups, were: oxygen-warm, 4.3 ± 1.5%; nitrogen-warm, 15.4 ± 2.5: oxygen-cool, 11.6 ± 1.7%; nitrogen-cool, 18.9 ± 3.0%. Similar results obtained by Edmondson and Meyer on sex-linked lethals induced in female embryos of the same treated material are reported separately (these Records). Our results, indicating that "reactivation" occurs more actively with oxygen, and at the higher temperature, probably represent a different mechanism from those obtained by others for oxygen and temperature with X-rays. A6.

*This work was supported by a National Cancer Institute, U. S. Public Health Service research grant.

MICKEY, GEORGE H. and JERRY L. BLOUNT, Northwestern University, Evanston, Illinois. Genetic evidence for somatic disjunction in Drosophila.* — Genetic tests involving both the X-chromosome and the third chromosome indicate segregation of homologues in somatic tissues following treatment of larvae with the vapor of glacial acetic acid. The phenomenon is delayed; that is, it occurs in the progeny of treated individuals, producing mosaic flies. It seems likely that it is a centromeric effect. The treatment probably inhibits the reproduction or orientation of the centromere so that sister chromatids move together to the same pole and homologous chromosomes are segregated to opposite poles. Somatic crossing over (although induced by acetic acid treatment) is essentially ruled out in the third chromosome test since all the recessive markers (ru h st p^p ss e^s) appeared in the mosaic area. Only two alternative explanations exist, both of which are highly improbable: (1) double crossover between st and p^p, one on each side of the centromere in a region only 4 units long, or (2) non-disjunction and survival of tissue monosomic for the third chromosome. Twin spots produced in the X-chromosome tests could be accounted for by somatic crossing over but their relative frequency argues for origin in double non-disjunction or somatic disjunction. L11.

MILLER, DOROTHEA S., BENSON E. GINSBURG and MILDRED Z. POTAS, University of Chicago, Chicago, Ill. Inheritance of seizure susceptibility in the house mouse (Mus musculus).** — Further studies have been made on the inheritance of susceptibility to sound-induced seizures in the mouse, through crosses of dba lines 1 and 2 (susceptible) to C57 black, sublines 6 and 10 (resistant), carried to the F_2 generation. In testing for seizure-susceptibility, each animal was exposed once daily for four successive days, starting at 30 days of age, and was tabulated according to the most severe reaction exhibited on four trials. On this basis, the incidence for the C57 black strain (sublines 6 and 10) was < 5%; that of dba line 1, 81%; and that of dba line 2, 98%. In F_1, all crosses involving dba-2 showed greater dominance than the corresponding crosses with dba-1; the C57-10 crosses showed a higher incidence than the corresponding crosses with C57-6. In F_2, the convulsive incidence was higher in crosses derived from C57-6 than in those derived from C57-10. All F_2 populations exhibited a lower seizure incidence than the corresponding F_1, except for dba-1 by C57-6, where virtually the same incidence was obtained in F_1 and F_2. — A sex difference in susceptibility is evident in several categories. In crosses involving C57-10, any sex differences that appear indicate a higher incidence in males. In crosses involving C57-6, the higher

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incidence, if any, is in females. In a number of crosses, reciprocal matings result differentially in sex differences in seizure incidence; in others, in a significant difference in total incidence. These differences in reciprocal crosses appear in a few categories in both F_1 and F_2. C12.

MITTLER, SIDNEY, Illinois Institute of Technology, Chicago, Ill. Influence of amino acids upon the appearance of tumors in tu^{50j} stock of D. melanogaster. — Tu^{50j} is a second chromosome recessive mutation which produces melanotic growths in the abdomen. The penetrance of tu^{50j} had been reported previously (Science 115: 271. 1952) to be dependent upon nutrition. The flies were raised on a vitamin-amino acid free medium consisting chiefly of glucose and sodium ammonium phosphate, and was inoculated with Hansenula anomala, H. saturnus or Debaromyces globosus, yeasts which require no vitamins or amino acids for growth. To the above minimal medium excess amounts of amino acids were added and the effect upon the penetrance of tu^{50j} was recorded. In all fourteen amino acids were tested. Tryptophane, lysine, and asparagine increase the percentage of flies that have tumors. Cystine, alanine, phenyalanine, and leucine decrease the penetrance of tu^{50j} while tryosine, glutamic acid, arginine, norleuicine, histidine, methionine, and threonine have no apparent effect upon tumor production. L9.

MOREE, RAY, State College of Washington, Pullman, Washington. A procedure for the detection and measurement of assortative mating. — Although it is commonly considered that assortative mating is unimportant in evolution, it does not necessarily follow that it is unimportant to the analysis of evolutionary changes occurring in an experimental population, especially when dominance is involved. Since its main effect is to vary zygotic ratios from those under panmixis, for a given gene frequency, it contributes to the inaccurate measurement of gene frequency unless it is detected and its effect measured. In the method here suggested a coefficient of assortative mating, c, is measured on the usual correlation scale; tests auxiliary to the main population experiment are set up to measure it. A population α AA, β Aa, γ aa is established in panmictic proportions and with equal gene frequencies. Since A is dominant, $\alpha + \beta = D$; $\gamma = R$. A second generation is reared and c is measured by comparing the two (allowing for selection and heterosis). Equations to measure c are derived for positive and negative assortative mating, based on all three genotypes. Those based on aa are: $(D_1 + cR_1)\beta_1^2/4D_1 + [1 - \alpha_1(1 - c)]R_1 = R_2$ (+ assortative mating), $(1 + c)\beta_1^2/4 + [(1 + c)D_1 - c\beta_1/2D_1R_1]R_1 = R_2$ (- assortative mating). Solving for c and simplifying according to experimental conditions gives, respectively: $c = 12(R_2 - 0.25)$; $c = 12(0.25 - R_2)$. Ordinary tests of significance can be applied without difficulty. When the degree of assortative mating is known from auxiliary experiments, corresponding adjustments can be made in interpreting the behavior of a cage

population. Limitations of the method, in addition to those already mentioned, are the assumption that mating is panmictic within phenotypes and that DxR and RxD are equivalent. B4.

MORGAN, WALTER C., University of Connecticut, Storrs, Conn. A new Crooked tail mutation in the mouse. — A new tail mutation, Crooked (Cd), discovered in 1948 in strain "A" mice, at the National Cancer Institute behaves as a dominant which is lethal, or semi-lethal, when homozygous. The tails of Cd/+ animals vary from those which are somewhat shortened with 4 or 5 angular kinks to phenotypically normal-tailed overlaps. Of 60 normal-tailed F_2 mice tested by matings with normal, 12 proved to be Cd/+. Genotypically normal-tailed mice mated to mutants have produced at birth, 221 mutants to 239 normal-tailed. Cd/+ x Cd/+ gave 275 mutants to 178 normal-tailed. Correcting for normal-tailed overlaps, the chi-square for 2 : 1 = .804, P = .39; for 3 : 1 x^2 = 10.02, P < .01. Of 253 F_2 mutants, reclassified at weaning, 38 were SMALL. No homozygotes have been found from 80 tested non-SMALL F_2 mutants. The SMALL mutants exhibit a marked retardation of growth and non-eruption of lower incisors. Other irregular anomalies occurring in the SMALL mice are: abnormal eyes (microphthalmia or anophthalmia), nervous head movements, kidney deficiency, and shortened appendages. These mice may represent the Cd/Cd class. No SMALLS have been discovered in a population of 329 weanlings of this strain produced from matings of other than Cd/+ x Cd/+. Preparturition autopsies of Cd/+ females fertilized by Cd/+ males, have disclosed pseudencephalic embryos similar to those reported by Bonnevie (1936). The hypothesis being tested is that Cd/Cd is sometimes lethal before birth, but may survive to produce the SMALL syndrome. C6.

MOSES, M. J. and G. YERGANIAN,* Brookhaven National Laboratory, Upton, N. Y. Desoxypentose nucleic acid (DNA) content and cytotaxonomy of several Cricetinae (hamsters).** — The European and Asiatic members of the sub-family Cricetinae are characterized cytologically by extreme differences in chromosome number: Mesocricetus (Cricetus) auratus, 2n = 38 (Koller, Muldal); or 2n = 44 (Husted et al, Matthey); Cricetus cricetus, 2n = 22 (Matthey); and Cricetulus griseus, 2n = 22 (Matthey, Sachs, Yerganian). The sex bivalents in these species are similar in morphology, heterochromaticity and nucleolar association during meiosis. — R. Matthey and R. B. Goldschmidt have discussed macroevolutionary mechanisms, such as chromosome fragmentation, duplication, inversions, translocations and polyploidy in establishing new genera. Heretofore, these mechanisms have been described in various invertebrates and many plants. No clear cytological

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evidence exists, however, to indicate which of these mechanisms may have been involved in the evolution of the Cricetinae and hence the evolutionary relationships of the included genera are uncertain. — In this study, the DNA content of nuclei was determined cytophotometrically in various tissues of the Syrian and Chinese hamsters, M. auratus and C. griseus, respectively. The preliminary data suggest that chromosome fragmentation and not duplication or polyploidy, took place in establishing the Syrian hamster as a systematic unit. This conclusion is based on the concept of DNA constancy and on the assumption that the basic chromosome number for the Cricetinae is 22. H4.

MULLER, H. J., Indiana University, Bloomington, Indiana. The standard error of the frequency of mutants some of which are of common origin.* — When the mutation frequency, p, must be determined from tests of cells which underwent varied amounts of proliferation, uncorrelated with their mutation rate, between the times of mutation and testing, some of the mutants have a common origin, occurring in "runs of different sizes. It is then illegitimate to apply the ordinary formula for s_p, the standard error of p, $s_p^2 = \frac{q \cdot p}{n}$, or $\frac{q \cdot P}{n^2}$, where $q = (1 - p)$, P is the absolute number of mutants ($= p \cdot n$), and n is the total number of tested germ cells. However, this formula can be modified, to suit cases where mutants of common origin can be identified as such. — Let r denote the size of a given run and I_r the number of independent mutations manifested in runs of that size. The number of mutants found in these runs is then $r \cdot I_r$, and P represents the sum of all the $r \cdot I_r$ products. What we wish to find, then, is the standard-error^2 of P/n, i.e. of the sum of $r \cdot I_r/n$. Assuming I_r itself to arise randomly, its standard-error must be the square root of $q \cdot I_r$. As the standard-error of $r \cdot I_r$ is r times that of I_r, the standard-error^2 of $r \cdot I_r$ is $r^2 \cdot q \cdot I_r$. Then, since P is the sum of all $r \cdot I_r$ values, and these are assumed independent, its standard-error^2 is q times the sum of all $r^2 \cdot I_r$ values. As p is P/n the desired formula is $s_p^2 = q \cdot \mathrm{Sum}(r^2 \cdot I_r)/n$. — This method has been applied to results from irradiation of early germ cells (Meyer and Muller, Edmondson and Meyer, these Records). B5.

MURRAY, MERRITT J., A. M. Todd Company, Kalamazoo, Michigan. Evolutionary trends and species barriers in three genera, Acnida, Amaranthus, and Mentha. — These groups, alike in being cross-pollinated and highly labile, are unlike in their manner of pollen transmission and method of survival, and almost wholly different in their development of species characteristics and species barriers. The wind pollinated amaranthaceous plants early exhibited three sex forms and an aneuploid change in chromosome number with the subsequent de-

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velopment of species differing in quantitative and qualitative factors. In contrast, evolution in Mentha has involved a series of changes in diploid chromosome number ranging from 18, 20 to 40, and from 24, 36, 48, 72 to 96. The fertile "pure species" and sterile "hybrids" of European authors are interpreted as balanced and unbalanced structural hybrids. The more than thirty intergeneric and four hundred interspecific combinations studied so far indicate that the two major genera involve species of divergent and separate origins, and that the generic boundaries are at best a convenient although artificial grouping of allied species. They further show that species barriers are both genetic and non-genetic of many sorts and that certain barriers are the consequence of adaptive evolutionary changes having a priori nothing to do with hybridization. A direct study of several species clearly demonstrates (1) that the qualitative genes of classical genetics play only a window dressing role in evolution, (2) that quantitative genes account for the major differences, and (3) that hybridization rather than mutation is often the principal modifying force. Taxonomic studies necessarily err in being unable to distinguish genetic differences from non-genetic effects of competition and environmental influences of soil, shading and the like, and (2) unnecessarily err in attempting to fit labile species with variability into a fixed notion derived from static species having negligible variation. B7.

MURRAY, MERRITT J., A. M. Todd Company, Kalamazoo, Michigan. Colchicine-induced tetraploids of fifteen species of Mentha. — Living plants of fifteen species of mints and their colchicine-induced tetraploids will be exhibited in support of the evolutionary trends described in the previous abstract. D6.

NANNEY, D. L., University of Michigan, Ann Arbor, Mich. Cytoplasmic Control of Nuclear Behavior at Conjugation in Tetrahymena. — Several strains of Tetrahymena, collected by Elliott, have been studied during the process of conjugation in an effort to determine the mechanisms controlling the complex reorganization processes. The general pattern of nuclear behavior corresponds closely to that reported by Maupas in 1889. Additional observations have been made on strains containing several micronuclei and on clones which have been centrifuged during conjugation. — Centrifugation results in the relocation of the nuclei in the cytoplasm at various stages of conjugation; correlated with, and presumably due to, this cytoplasmic relocation are alterations in the subsequent nuclear behavior and nuclear differentiation. Specifically the following types of aberrations have been noted: 1) An alteration in the number of new nuclei formed and 2) a transformation of presumptive micronuclei into macronuclei and of presumptive macronuclei into micronuclei. — In light of two facts, that nuclear events are specifically localized in the cytoplasm, and that an alteration in cytoplasmic position results in altered behavior and development, it is suggested that the cytoplasm controls to a large extent

the nuclear events in conjugation. The mechanism involved in placing nuclei at specific cytoplasmic positions is also discussed. K10.

NEWCOMBE, H. B., Atomic Energy of Canada Ltd., Chalk River, Ontario. The copy-error concept of gene mutation. — There are two commonly held concepts of gene mutation, (a) as a molecular change caused by mutagenic chemicals within the cell and (b) as an inaccurate self duplication of the gene. The first has recently been expanded to include all mutations of whatever origin, while the second has been considered mainly in relation to spontaneous change. In addition, the evidence from spontaneous mutation would appear to be about equally divided for and against the latter concept, mutation being in some experiments a function of division and in others a function of time and independent of division. However, both conclusions seem valid under the conditions of the experiments, and the apparent conflict could be resolved by combining the two concepts, the amount of mutagen formed in the cell determining the likelihood of a copy error at gene duplication. Induced mutations seem from our experiments with bacteria to be delayed until about the time of the first gene division. Thus, although neither spontaneous nor induced mutations have as yet been demonstrated to occur during gene duplication, the possibility is not ruled out by any of the evidence from either source, and all of the phenomena associated with mutation could be interpreted on the combined concept of chemical mutagens (either natural or artificial) acting to produce inaccuracies in gene copying. If this is the case, some effective post-treatment for radiation induced genetic damage may yet be found. K5.

NORMAN, AMOS, Atomic Energy Project, University of California, Los Angeles, Calif. Radiation induced phenocopies in bacteria.* — Ultraviolet radiation induces the formation of phenocopies in A. aerogenes characterized by their inability to grow in media containing as sole carbon source any one of the following compounds: acetate, citrate, succinate, lactose, or rhamnose. Each of these compounds is an adaptive substrate for cultures grown in glucose; this suggests, therefore, that the radiation blocks the adaptive mechanism in some way. Such an hypothesis is supported by the finding that the formation of phenocopies incapable of utilizing any one of these compounds for growth can be suppressed by adapting the bacterial population to that compound prior to irradiation. An analysis of the survival curves suggests that the formation of phenocopies is a consequence of some alteration in the bacterial nucleus. — In general the ability to utilize a given carbon source is not independent of the ability to utilize another source. For example, phenocopies incapable of growing on citrate cannot grow on succinate, but those capable of growing on succinate can also grow on citrate. K6.

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OSTER, IRWIN I., Indiana University, Bloomington, Indiana. A developmental study of lethal point mutations induced in spermatozoa of Drosophila melanogaster by ultraviolet.* — To throw light on the manner of action of lethal mutations, the time of death and possible visible abnormalities of 55 recessive sex-linked lethals were studied. These had been induced by ultraviolet treatment of spermatozoa and found to entail no detectable chromosome changes (Valencia and McQuate, these Records, 1951). We found that 7 lethals killed the embryos just before hatching from the egg case, while 27 lethals, the largest group, killed just after this, i.e., during the first larval instar. Other periods in which lethals were found to exert their killing action were just before pupation (9 lethals), just after pupation (9 lethals), and—less often—just before eclosion (3 lethals). None exerted its main killing effect during the 2nd instar. Eight of the lethals were found to cause visible morphological abnormalities—including one causing melanotic tumor formation. We interpret the marked clustering of effects at these critical periods as being caused—not by development per se being more active then, but by the fact that these are the times when many new physiological processes, on the combination of which the life of the individual depends, are set into active operation for the first time in its life. Hence mutant genes not functionally active previously are especially likely to produce their deleterious effects at such a stage. In the developmentally most active periods—those of the early and middle-stage embryo—life is evidently maintained by physiological processes which depend largely on gene products of the preceding generation. L4.

OWNBEY, MARION, State College of Washington, Pullman, Washington. Cytoplasmic inheritance and reciprocal amphiploidy in Tragopogon. — Interaction between the cytoplasm of T. pratensis and the chromosomes of T. porrifolius produces striking differences between reciprocal F_1 hybrids between these species. In T. pratensis ♀ x porrifolius ♂ F_1, the ligules are only about half the length of the involucral bracts and are brownish in color with conspicuous yellow tips. In the reciprocal, the ligules are nearly as long as the bracts, are deep red, and lack yellow tips. In the F_2, long-liguled plants produce only long-liguled offspring. Short-liguled plants produce offspring with ligules varying from shorter than in the F_1 to appreciably longer. Ligule color is highly variable in the F_2, but yellow tips are found only among progeny of the yellow tipped F_1. When T. porrifolius is crossed with T. dubius, all offspring are long-liguled and no reciprocal differences have been detected. In T. pratensis ♀ x dubius ♂ F_1, all progeny are short-liguled. The reciprocal has not been obtained, but long-liguled apparent F_1's are found in the wild and the corresponding long- and short-liguled races of T. miscellus (amphiploid dubius x pratensis) occur spontaneously. When these are crossed, hybrids cannot be de-

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tected among the progeny, but when crossed with T. mirus (amphiploid dubius x porrifolius), short ligules appear when the short-liguled race serves as the pistillate parent. J7.

PATAU, KLAUS, Program in Cytology, Department of Botany, University of Wisconsin. The DNA content of nuclei in root tips of Rhoeo discolor. — Absorption measurements of Feulgen-stained nuclei in longitudinal sections of Rhoeo roots (Navashin fixation) were made by means of a Farrand phototube at λ = 5461 Å. In the meristem the nuclear growth between telophase and prophase is correlated with (and there is evidence that it may be determined by) the cell size. The volume of nuclei at a given stage varies, therefore, considerably between different cell rows. Within a row the DNA content is closely correlated with the nuclear volume: up to a certain size (e.g. in one row ca. 400 μ^3) the simple diploid amount, 2C, is present. In nuclei of a certain, subsequent, range of volumes (e.g. ca. 400-600 μ^3) a positive regression of DNA content (leading from 2C to 4C) on volume is found. The frequency of intermediates (between DNA classes) in the actively dividing meristem indicates a duration of the DNA synthesis of several hours. Prophase starts, in slightly largely nuclei, only after the conclusion of this synthesis. No intermediates have been observed in the elongation zone. Here in certain cell rows either 2C or 4C nuclei greatly predominate; in other rows both occur in similar frequencies. It is concluded that DNA synthesis and onset of prophase are independent processes to the extent that either one can be blocked without immediate inhibition of the other process. After the end of the mitotic period the volumes of 2C and 4C nuclei increase slowly and retain, during cell elongation, approximately their initial average ratio of, roughly, 1 : 1.5. In the elongation zone 8C nuclei occur only as rare exceptions. None has been seen in the meristem. Higher parts of the root have not yet been investigated. T10.

PATTERSON, E. B., California Institute of Technology, Pasadena, California. The use of functional duplicate-deficient gametes in locating Genes in maize. — Adjacent-1 disjunction from a heterozygous translocation complex yields spores duplicated for one arm of the cross configuration and deficient for the opposite arm. A functional duplicate-deficient egg when fertilized by a sperm of normal chromosome constitution gives rise to a plant having one chromosome segment in triplicate and another segment represented singly. When appropriately testcrossed, a duplicate-deficient plant gives modified trisomic ratios for genes carried in the triplicated segment and a hemizygous test for genes in the deficient segment. — Preliminary studies of duplicate-deficient types involving the short arm of chromosome 2 show that lg_1 is located a few chromomeres from the tip and that gl_2 and B are in the distal half of the arm. The gene sk_1 appears to be about one-third of the way out in the arm. In chromosome 9, C is at a position about three-fourths of the way out on the short arm and sh_1 is nearby.

Transmissible duplicate-deficient types involving other chromosome arms have also been studied. — Functioning of duplicate-deficient eggs has been highly variable and appears to be strongly influenced by environmental conditions. In these studies there have been no confirmed instances in which duplicate-deficient pollen grains have functioned. M5.

PAULEY, S. S., Maria Moors Cabot Foundation for Botanical Research, Harvard University, Petersham, Mass. Photoperiodic growth response in Populus. — Studies of ecotypic variability in Populus species have revealed the important role of day length in the annual termination of growth activity. Long-day ecotypes native of northern latitudes consistently go into abnormally early dormancy when grown in the shorter days of southern latitudes. Such reactions occur in spite of the fact that otherwise favorable growing conditions may persist for several months in the short-day environment. Conversely, short-day ecotypes native of southern latitudes, when grown in the longer days of northern localities, fail to stop growth before the first frosts of autumn and in consequence are killed back. Hybrids produced from crosses of northern and southern ecotypes terminate annual growth activity at times intermediate to the dormancy dates of the parents when grown together in the same day length. Exceptions to this general behavior are strikingly demonstrated by the high-altitude, short-growing-season ecotypes of trembling aspen native in regions of short day, such as the central and southern Rocky Mountains in the United States. When transplanted to test plots at the latitude of Boston these ecotypes characteristically demonstrate early seasonal growth termination, in a manner comparable to the reaction given by ecotypes from the long days of high latitudes. If southern Rocky Mountain forms of aspen represent migrants from the north, the preadaptability in growth pattern of these types to the short season of the high altitudes may have been highly beneficial to the species' southward migration. On the other hand, if migration were northward, the preadaptability of such high altitude southern types to long days would have been equally advantageous. T11.

PERKINS, D. D., R. W. BARRATT, and DOROTHY L. NEWMEYER, Stanford University, Calif. Mapping functions for tetrads, and their application to Neurospora. — Functions proposed by various workers for relating single strand recombination frequencies to map distance do not provide an adequate theoretical basis for arriving at analogous mapping functions that would relate the frequency of tetratype tetrads or 2nd division segregation tetrads to map distance. By making simple assumptions regarding the effect of interference on the proportions of bivalents having different numbers of exchanges, mapping functions can be derived for any specified interference intensity. Pairs of corresponding functions can be obtained that are applicable to single strands and to tetrads respectively. — Interference assumptions that give a

single strand function consistent with data from Drosophila have been used to derive the corresponding tetrad function for application to Neurospora. The uncorrected maps and confidence limits described by Barratt, Newmeyer and Perkins (see abstract) have thus been modified to correct for multiple crossovers, assuming that interference in the two organisms is similar. G12.

PETERSON, PETER A., University of Illinois, Urbana, Illinois. Effect of temperature on the mutation rate of a mutable locus in maize. — One method of analyzing the nature of mutation is to study the effect of temperature differences on a mutable locus. Previous investigations of this kind on unstable genes, as in Portulaca (Fabergé and Beale), and in the Dt-a_1 relation in maize (Rhoades), have shown that an increase in temperature is accompanied by a decrease in mutation rate. — In contrast to this, studies with a mutable pale green gene that mutates from recessive pale green(pg) to dominant green (Pg) show that there is an increase in mutation rate with increase in temperature. Using seven uniformly mutating families, counts were made, at comparable stages of growth, of green stripes in the first 3 leaves of mutant seedlings grown at 16°C and 29°C. The results show that in all seven families the increase in mutation rate in the seedlings treated at 29°C ranged from 2.3 to 17 times the rate in the seedlings at 16°C. (This range, from 2.3 to 17, is attributed to different genetic backgrounds.) — In addition, it is evident that increases in mutation rate are greater in the third leaf than in the second leaf, greater in the second than in the first leaf of the seedlings. These differences in comparisons between leaves are tentatively explained as due to the fact that a greater part of the development of successively younger leaves is effected by the different temperatures. M6.

PLAINE, HENRY L. and BENTLEY GLASS, Johns Hopkins University, Baltimore, Md. The role of oxygen concentration in determining the effectiveness of x-rays on the action of a specific gene and on tumor formation in Drosophila melanogaster. — X-ray treatments of embryos of the suppressor-erupt stock block the action of a specific suppressor gene (Su-er) and allow the suppressed mutant gene erupt (er) to manifest itself in the adult stage. This stock also shows a high incidence of melanotic tumors (81.7%) induced by x-rays. — When embryos were irradiated with 1000 r units at 10 $\pm$ 1 hrs. in concentrations of 0% O_2, 10% O_2, 20% O_2, and pure oxygen, there was, with increasing oxygen concentration, 1) increased inhibition of the action of the suppressor gene, with corresponding increase in the expression of erupt; 2) increased mortality in the larval and pupal stages; 3) increased duration of development, with delayed pupation; and 4) increased incidence of tumors as well as increased number of tumors per affected larva. There is no observable effect when embryos are

exposed to various O_2 concentrations without being x-rayed. The incidence of erupt and the incidence of tumors both increase significantly and linearly from O to 20% O_2, above which concentration there is only a small, though significant, further increase. The same treatment applied to an Oregon-R inbred strain gives similar results but at a much lower level. — Mortality, extended and delayed development, and induction of melanomas all parallel the effects of x-rays on the suppression of erupt at given oxygen tensions; but further work will be required to make clear the nature of the relation between the suppressor of erupt and the parallel effects. A5.

PUNNETT, HOPE H., University of Illinois, Urbana, Ill. An aberrant fragment chromosome in maize. — A rod-shaped fragment chromosome with interstitial centromere arising from a telocentric short arm of chromosome 5 in Zea mays has been investigated. The rod, which includes the loci A_2 and Bm_1, has been found to give rise to ring-shaped chromosomes with regular frequency. Rates of ring formation observed are 8.2% in male gametes and 1.2% in female gametes. Since the rings may contain either one or both of the two loci, crossing over is suggested as a factor in their origin, and the sex difference noted above may reflect the difference in crossover values for male and female gametes which is known for this region of the normal chromosome 5. M7.

RAM, T. and F. B. HUTT, Cornell University, Ithaca, N. Y. The physiological basis for genetic resistance to Salmonella pullorum in the fowl. — Resistance to this organism, which increases greatly during the first week after hatching, was found in a series of experiments to be dependent upon body temperature rather than upon the proportion of lymphocytes, although post-natal rises in both of these coincide with the rise in resistance. Interfamilial differences in mean body temperature were significant and were related to corresponding differences in mortality following inoculation with S. pullorum, the correlation between temperature and mortality being -0.49. These same families did not differ significantly in proportion of lymphocytes, and the correlation between that proportion and mortality was only +0.14. Correlations between body temperature and proportion of lymphocytes were low and insignificant. When chicks were brooded at 40°C., their temperatures and resistance were both raised, but their lymphocytes were fewer, in comparison with chicks brooded at lower temperatures. As in previous studies, White Leghorns were more resistant than Rhode Island Reds, and crossbreds, which had lower counts of lymphocytes than the Rhode Island Reds, proved to be more resistant than the latter. Rate of basal metabolism was higher in high-temperature families than in those with low temperature. — Since the rate of phagocytosis is greatly accelerated with any rise in temperature, it is considered

probable that the higher temperatures and superior thermoregulatory mechanism of the resistant chicks exert their beneficial effects, in part at least, by accelerating that process. B9.

RAPER, JOHN R. and JAMES P. SANANTONIO, University of Chicago, Chicago, Ill. Heterokaryotic mutagenesis in the tetrapolar fungus, Schizophyllum. — Matings of mycelia having common incompatibility alleles at the "A" locus yield reciprocally constituted, stable, infertile heterokaryons. In cultures of aging heterokaryons of this type, mutants occur at a frequency ranging from 10^{-9} to 10^{-5} as compared to an upper limit of 10^{-11} in the normal component mycelia. The mutants are monokaryotic, roughly equally distributed between the two mating types of the heterokaryotic components, and comprise about eight distinct morphological types, each so far tested differring from normal by a single affected locus. Of 60 morphological and biochemical mutants isolated from normal monokaryotic mycelia irradiated with UV, only two individuals correspond to heterokaryotic mutant types. The mutation frequency in heterokaryons varies within wide limits according to the genetic histories of the mated mycelia. Mutations of the same types as those appearing in heterokaryons may be induced in normal monokaryotic mycelia when the latter are grown for 10-20 days in the cell-free filtrate of aged, common-A heterokaryons. G7.

RATTY, F. J., JR., University of Utah, Salt Lake City, Utah. Gene action and position effect in duplications of the white-Notch region of Drosophila melanogaster. — The ability of six different short duplications, three of the white and three of the w-N region, to cover sex-linked lethal mutations within their limits was studied. Each duplication is inserted into an autosome. Three of these duplications were produced from + chromosomes, two from w^{m4}, and one from rst^{3}. Four of the duplications show mottling for the visible loci present, indicating association with heterochromatin; two do not. — All six are able to cover w lethal or N mutations. Forty-five w deficiencies and 40 N mutants were crossed with these duplications. The mottled duplications cover as many w deficiencies or N mutants within their limits as the non-mottled ones do. Moreover, tests of 20 w-N deficiencies gave similar results. The inability to cover some of the lethals within the limits of the duplications is not a function of the duplication, but rather is an intrinsic property of the tested chromosome (i.e., independent lethals). Fertile covered N males are usually non-N in appearance, but transmit the N phenotype unchanged to their daughters. In a few cases the covered males are themselves N, but these are invariably sterile, although non-N duplication males may also be sterile. The mosaicism exhibited by the mottled duplications affects only the phenotypic expression of the visible loci concerned; there seems to be no position effect on lethality at the Notch or any other locus in these insertional dupli-

cations. However, sometimes the production of covered males is low, particularly with the mottled duplications. T12.

RAUCH, H., University of Massachusetts, Amherst, Mass. Strain differences in liver patterns in mice. — Mice of three inbred strains, C_{57} Black, dba, and A-albino (Bar Harbor), were examined for variations in liver lobation. From observations of from 90 to 279 animals of each strain, thirteen patterns were determined. These variations are generally in the direction of fission rather than of fusion of lobes. Statistical analysis reveals significant differences in the incidence of liver patterns between the sexes within a strain and between strains. In general, there is less variability among females of a strain than among males of the same strain. — Crosses made within and between strains and tested for parent-offspring correlation indicate a marked mother-daughter correlation. The lack of correlation for the males confirms the greater variability observed for this sex. — Fertilized eggs of one strain were transplanted into the uterus of another and allowed to develop. Among the female progeny of such transplants (males were not observed), a shift in pattern incidence toward that of the host strain is noted. This suggests that the uterine environment is a major factor in the determination of the liver pattern. T13.

RICK, CHARLES M., University of California, Davis, California, and BARTON, DONALD W., New York State Agricultural Experiment Station, Geneva, New York. The primary trisomics of Lycopersicon esculentum. — The objective of this investigation was to identify for each of the tomato trisomics the corresponding pachytene chromosome and genetic linkage group. As far as comparisons permit our observations agree with the previous findings of Lesley. All trisomic plants were obtained from the progeny of triploids of the var. San Marzano, 45.2 percent of 546 seedlings having a single extra chromosome according to root-tip counts. The trisomics could be classified readily into 12 morphological types that are sharply distinguished by such features as plant habit, fruit shape, and color, size, and shape of leaf. — The pachytene chromosomes, which are well differentiated, have been identified with certainty for each of the trisomics; figures for the twelfth are not clear but it appears very likely that the remaining chromosome corresponds to the remaining trisomic type. Frequencies of appearance in triploid progenies and also frequencies of transmission in trisomic families differ greatly for the various trisomics and do not bear any simple relation to pachytene chromosome length. — Genetic analysis is greatly facilitated by the ease of identification of the trisomic types in F_1 and segregating progenies. Genes in the linkage groups I-VIII have been tested against nine trisomics. Trisomic segregations have been observed for the following linkage groups: I (d_1), V (wt), and VII (H). Genes of VI (l) and VIII (a_2) segregate in trisomic fashion for the

same trisomic type and are therefore carried on the same chromosome. The gene wd, whose linkage relations had not previously been investigated, corresponds to a trisomic that is not related to groups I-VIII and must therefore represent a new linkage group. D7.

RIETSEMA, J., Genetics Experiment Station, Smith College, Northampton, Mass. The presence of indole-3-acetic acid in ovular tumors of incompatible Datura crosses and its possible role as an embryo inhibitor.* — Addition of indole-3-acetic acid to cultures of Datura stramonium embryos in vitro causes inhibition between 1 and 0.01 p.p.m., higher concentrations being lethal and lower ones having no effect or a slight stimulation. Elongation of hypocotyls is more inhibited than that of cotyledons, which show much increase in width at base. At 1 p.p.m. no meristem can be distinguished between the cotyledons. Malt extract in the medium tends to prevent this inhibition. — Extracts of ovular tumors from the cross Datura innoxia x D. discolor produce a similar effect on D. stramonium embryos in culture. Therefore the auxin content of ovular tumor extracts was tested with the standard Avena technique. Amount of auxin varies between 0.004 and 0.013 mg per gram tissue, assuming that all auxin is indole-3-acetic acid. The average weight per tumor is approx. 0.5 mg. Boiling in 0.02 n NaOH for 10 minutes increased the auxin yield 2-10 times. The nature of the growth substance was determined by paper partition chromatography with phenol-H_2O sat. and butanol-H_2O sat. as solvents. Elution of the paper and subsequent tests with Avena coleoptiles showed that the Rf value of the auxin corresponded to that of indole-3-acetic acid. A small fraction proved to be of a different nature. The location of the embryo inhibitor on the paper was the same as that of indole-3-acetic acid. It is, therefore, tentatively concluded that at least one of the causes of embryo abortion is indole-3-acetic acid located in ovular tumors. D8.

RILEY, H. P., Oak Ridge National Laboratory, Oak Ridge, Tenn. Preliminary report on the effect of certain chemicals on radiation damage to chromosomes. — Onion roots from 1 to 3 cm. long were immersed in various solutions for 30 min., removed and irradiated with gamma radiation in air, and then returned to the solutions for ten minutes. They were then transferred to water where they remained for three or four days at which time slides were made for a study of chromosome aberrations. The solutions in which they were immersed were .02M, .002M, .0002M, .00002M, .000002M, and .0000002M concentrations of sodium hydrosulfite or 2, 3 dimercaptopropanol (BAL). Roots kept in water before and after irradiation were used as controls. Doses

*This project was supported by a grant from the National Cancer Institute, U. S. Public Health Service.

up to 280r produced fewer aberrations than in Tradescantia microspores but higher doses tended to inhibit or retard mitosis. At 240r there were 0.083 interchanges and 0.147 deletions per cell for the roots in water and comparable figures were found for the same dose combined with .00002M to .0000002M concentrations of both chemicals, but the aberration frequency was reduced to about one-half in the stronger concentrations; in one experiment roots in .02M sodium hydrosulfite were killed. A dose of 420r practically completely inhibited mitosis in the roots in water and in .0002M sodium hydrosulfite, but about 1% of the cells were in division in the roots treated with .02M and .002M sodium hydrosulfite before and after radiation. This preliminary study indicates that certain concentrations of these two chemicals reduce the damage to onion root tip chromosomes caused by gamma radiation. E1.

RIS, HANS, University of Wisconsin, Madison, Wisconsin. The sub-microscopic structure of chromosomes. — Lampbrush-chromosomes of Triturus, Necturus and Amphiuma were dissected from the egg nucleus onto a formvar film on a microscope slide, fixed in osmium tetroxide, shadowed with uranium and mounted on a screen for the electron microscope. Pachytene chromosomes from spermatocytes of the grasshopper Chorthophaga and the hemipteran Oncopeltus were isolated by squashing testes fixed with Carnoy and stained with Feulgen on a slide coated with parlodion. The slides were shadowed with uranium and suitable regions of the film mounted on a wire screen. Electron microscopy showed that these chromosomes are made of sub-microscopic fibrils, each fibril forming a small-gyred helix. A bundle of tightly packed microfibrils forms the chromonema visible in the light microscope. The microfibrils in the lampbrush chromosomes of the 3 salamanders are about 500 Å thick, those in the chromosomes of the 2 insects approximately 600 Å. Yasuzumi, Odate and Ota (Cytologia 16: 233) have described recently microfibrils in the salivary gland chromosomes of Drosophila that are 500 Å thick. The chromonemata in the lampbrush chromosomes of different salamanders vary in thickness and this is related to the DNA content of the chromosome set. Amphiuma with highest DNA per chromosome set has the thickest chromonemata, next Necturus followed by Triturus. Since all have the same elementary unit they must differ in the number of microfibrils that make up the chromonema. These preliminary studies suggest that animal chromosomes consist of sub-microscopic elementary units, spiralled microfibrils, that in the species analyzed so far are from 500-600 Å thick. Variations in thickness of chromonemata from one species to another seem to be due to difference in number of microfibrils that compose the chromonema. D9.

RIZKI, M. T. M., Columbia University, New York, N. Y. Ontogenetic distribution of genetic lethality in Drosophila willistoni. — Spontaneous and X-ray induced recessive autosomal lethals of

D. willistoni were classified according to the stage or combination of stages at which death occurs. The ontogenetic distribution pattern of 24 spontaneous lethals extracted from natural populations was: 1 egg lethal (E), 2 egg-larval (E/L), 16 larval (L), 2 larval-pupal (L/P), and 3 pupal lethals (P). Similarly 29 newly arisen spontaneous lethals obtained from laboratory stocks were distributed as follows: 1 E, 4 E/L, 19 L, 4 L/P, 1 P. A sample of 19 X-ray induced lethals was distributed: 6 E, 3 E/L, 7 L, 0 L/P, 3 P. Spontaneous lethals most frequently cause death in the larval stage although some egg and pupal lethals also occur. X-ray induced lethals cause death in the egg stage relatively more frequently than do spontaneous lethals. — The problem of sensitive stages of ontogeny and their evolutionary aspects as revealed by the distribution of time of death due to lethal factors will be discussed. L7.

ROMAN, HERSCHEL, University of Washington, Seattle, Wash. Polyploidy and phenotypic ratios in Saccharomyces. — Clones of Saccharomyces that were originally haploid develop into mixtures of haploid and diploid cells. The diploids within a clone are of two kinds, either heterozygous or homozygous for the mating type alleles. The heterozygotes, which arise as a result of mutation at the mating type locus, sporulate readily and give 4-spored asci in which the mating type alleles segregate 2 : 2. The homozygotes, which do not sporulate, mate with haploid and diploid cells of opposite mating type. The triploid and tetraploid cells thus obtained produce asci which exhibit phenotypic segregations expected of their polyploid origin. If markers other than mating type are used, crosses between homozygous dominant and homozygous recessive diploids yield asci with 4 : 0 (4 dominants to 0 recessives), 3 : 1, and 2 : 2 ratios. Crosses between heterozygous and homozygous recessive diploids give 2 : 2 and 1 : 3 asci. Crosses between clones that are haploid-diploid mixtures sometimes give 0 : 4 asci; these in each case have turned out to be the result of the sporulation of cells heterozygous for mating type and present in the parental clones prior to the cross. Thus it is possible, in this material, to trace the origin of the various ratios to polyploidy or to mutation at the mating type locus. — The triploids, unlike the diploids and tetraploids, are highly sterile. The sterility evidence provides a basis for the assumption that there are at least four chromosomes in the haploid complement of the strains under investigation. — The mating system in these strains is largely heterothallic; however, evidence has been obtained that fusions of cells of like mating type occasionally occur. G6.

ROTHENBUHLER, WALTER C., JOHN W. GOWEN, and O. W. PARK, Iowa State College, Ames, Iowa. Five mutant genes in honey bees (Apis mellifera L.). — The wild-type eye color, black, is changed to white by three different genes. They are designated ivory (i), cream (cr), and snow (s). It has not been possible to see consistent distinguishable differences in the phenotypic expressions of these three

genes. A fourth gene, chartreuse (ch), is expressed as a yellow-green eye color in newly emerged bees. As the bee ages, the color changes, beginning in the lower part of the eye, to a reddish olive-green and finally to a reddish brown. These genes are recessive and non-allelic. Ivory, cream and snow are each epistatic to chartreuse. No evidence for linkage has been found between ivory and cream, ivory and snow, ivory and chartreuse, and cream and chartreuse. Tests for linkage of the genes in the other two combinations are in progress. — A fifth mutation, called Droopy (D), affects the wings. Instead of projecting directly caudad and on a level over the back, the wings are held at varying horizontal angles from the axis of the body and droop from the level of their attachment. Affected individuals cannot fly. This gene behaves as a dominant in females. No Droopy males have been obtained. Whether Droopy is lethal or unexpressed in males is now under investigation. — These five genes apparently mark five different loci in honey bees. B1.

RUSSELL, ELIZABETH S., JANE L. COULOMBRE, and ELIZABETH FEKETE, Jackson Memorial Laboratory, Bar Harbor, Maine. Contributions of Studies of gonad development and function and of hair spotting pattern to analysis of the W-series pleiotropism. — Although the action of the W and W^V alleles in producing macrocytic anemia is coming to be well understood as a persistent metabolic error in blood formation appearing first in embryonic life, the relation of the blood defect to sterility and pigment reduction found in the same genotypes has not been determined. Studies of gonads contribute to this question in three ways. (1) The sterility of W^VW^V, and WW is due to deficiency in number of germ cells and in differentiating capacity of germinal tissue present. (2) The gonad defect may be present as early as the anemia, since 13-14 day anemic embryos show reduced numbers of primordial germ cells. (3) Transfers of embryonic gonads into a neutral non-anemic site (castrate male spleen) allows autonomous development of ovary and testis but does not enhance the development of WW and W^VW^V germ cells. — Studies of spotting-pattern generalize the peculiarities of order of effect in the series. On four different color backgrounds W^Vw has significantly larger white areas than does Ww. In all tissues investigated the order of effect is ww = or > Ww = or > W^Vw >> W^VW^V > WW^V = or > WW. — If W-gene action is limited to a single tissue with secondary consequences at remote sites, this action must occur before the 13th day of embryonic life. It is at least as probable that the same type of local gene action independently alters the differentiation of three different tissues. D10.

RUSSELL, LIANE BRAUCH and MARY H. MAJOR, Oak Ridge National Laboratory, Oak Ridge, Tennessee. A preliminary report on radiation-induced presumed somatic mutations in the house mouse. — In an attempt to obtain somatic mutations at specific loci, embryos heterozygous for several pigment genes were irradiated, then raised

for observation of coat color. It was first determined that the developmental stage at which the number of pigment precursor cells is sufficiently large to make the experiment practicable, yet sufficiently small to produce spots of perceptible size is day 10 1/4 postconception. Doses used were 150r, 100r (for most of the irradiated group), and Or. — C57BL females are mated either to NB males to produce aa Bb CP/c^{ch}p DSe/d se embryos, or to C57BL males to produce aa BB CP/CP D Se/D Se embryos. The latter group serves as control against other effects of irradiation which might yield spots and also provides information on dominant pigment mutations anywhere in the genome. — In (C57BL x NB)F_1, 10 of 176 irradiated animals, and 1 of 202 nonirradiated, had colored spots. Measurements of the approximate proportion of total mouse surface occupied by the spot reveal that from 42 to 375 (average 158) pigment precursor cells were present at the time of irradiation (assuming all contributed equally to the adult coat); but, because of the diffuse nature of most spots, exact determination of somatic mutation rates for the loci in question will be difficult. Distribution over the body of the spots obtained is apparently random. — In C57BL, 2 of 102 irradiated animals, and 0 of 141 nonirradiated, had colored spots. Incidence of the small white belly spot occurring in C57BL is increased from 6.4% in controls to 26.8% with 100r. C10.

SAEZ, FRANCISCO A., Departamento de Citogenetica, Instituto de Investigacion de Ciencias Biologicas, Montevideo, Uruguay and Department of Zoology, Columbia University, N. Y. Cytological study of some South American species of the genus Hypochaeris (compositae). — Three South American species (Hypochaeris tweediei, H. microcephala, and H. megapotamica) each having 8 somatic chromosomes were studied. An evolution to the asymmetry of the caryotype is evident because all the elements are submetacentric, with an index of asymmetry rather high. The relative dimensions of the chromosomes show differences in size among the pairs of the genomes of the three species, each one having two long (A and B) and two short (C and D). — According to the caryotype of the three species structural modification must have developed along their evolution, with a reduction in the number of chromosomes so that the studied species are evolutioned forms of the genus and probably of the subtribe Leontodontinae. H10.

SAEZ, FRANCISCO A., Departamento de Citogenetica, Instituto de Investigacion de Ciencias Biologicas, Montevideo, Uruguay and Department of Zoology, Columbia University, N. Y. The cytology of Sorghum almum Parodi. II. The meiotic chromosomes. — Meiotic chromosomes of Sorghum almum Parodi, a new species with 40 somatic chromosomes, presents multivalent associations during the diplotene, diakinesis and metaphase I. Multivalent configurations support the interpretation of Saez and Nuñez (1943) that S. almum is an allopolyploid. Morphology and relative size of chromosomes of both parents are very much alike so it has been impossible to obtain direct evidence upon the

constitutions of the pairs of bivalents and the multivalent associations as well as to determine their origin. — It is suggested that pairing is accomplished by chromosomes belonging to the same set of gamete by autosyndesis, but due to some homology between the sets of both parents, it is possible that the multivalent associations would have also been produced by allosyndesis. H11.

SAWIN, P. B. and D. D. CRARY, Roscoe B. Jackson Memorial Laboratory, Bar Harbor, Maine. A second achondroplastic gene in the rabbit. — A second gene (da) inducing a recessive achondroplasia recently occurred in the New Zealand White breed of domestic rabbit in a commercial rabbitry in California. Unlike the achondroplasia (ac) described by Pearce, which is lethal at birth when homozygous, the (dada) individual is viable though apparently sterile. Among 68 animals resulting from known (Dada) parents, 18 were abnormal. Outcrosses of heterozygotes with unrelated races have produced 76 unaffected young. At birth (dada) animals are distinguishable externally only by an exceedingly minute double fissure at the inner base of the ear, between which by the 6th day develops a small distinct papilla. The head in some individuals is noticeably broader. X-rays show abnormality only in the scapula. As the animals mature, there is progressive development of abnormality. The head becomes broader and triangular, with a wide and characteristic drooping ear carriage, and well developed 1/4 inch ear papilla. Serial roentgenograms show that growth in the limbs, which at birth are apparently normal, is retarded from the time of first appearance of the epiphyses and is most pronounced in the femur, scapula and innominate bones, being especially centered around the acetabular, knee and shoulder joints. The innominate and scapula are cancellous with unusually large marrow cavities. These joints progressively enlarge from over growth of cartilage and numerous exostoses. Failure of development of the bony rim of the acetabulum, so as to enclose the head of the femur, may lead to early dislocation and bizarre deformity in the more seriously affected individual. The distal extremities are essentially unaffected. C2.

SCHWARTZ, DREW, Oak Ridge National Laboratory, Oak Ridge, Tennessee. Evidence for sister-strand crossing over in maize. — A large ring chromosome involving almost the whole of chromosome 6, the nucleolar organizer chromosome, was induced by X irradiation of maize pollen. Gametophytes possessing the ring and thus deficient for the chromatin in the terminal regions of chromosome 6 are viable. Crossing over in the heterozygote between the ring and its homologous rod chromosome results in bridges in both A_I and A_{II}. Cytologically it is possible to recognize two double crossover types. A four-strand double exchange gives rise to a double bridge in A_I; three-strand double (type b) in which a rod chromatid is involved in both exchanges gives a single bridge in both A_I and A_{II}. The frequency of single bridges in A_{II} was found to be approximately 3x the frequency of double A_I bridges.

It was possible to rule out negative chromatid interference. The data indicate that approximately 50% of the single exchange bivalents, which theoretically should give rise to single bridges in A_I only, also form single bridges in A_{II}. This can be accounted for by sister-strand crossing over. A single non-sister crossover associated with a sister-strand crossover in the ring will give rise to the same anaphase configuration which results from a three-strand double crossover of type b. The proportions of the four classes of anaphase configurations resulting from double non-sister crossovers are not changed by the occurrence of sister-strand crossing over. The high frequency of dicentric double ring formation in meiosis also suggests sister-strand crossing over. M8.

SEARS, E. R., U. S. Department of Agriculture and University of Missouri, Columbia, Missouri. Homoeologous chromosomes in Triticum aestivum. — The three genomes of hexaploid wheat consist of seven chromosomes each and are designated A, B, and D (or C), respectively. Genome A comes from diploid Triticum and D from Aegilops squarrosa, with the source of B unknown. The three genomes presumably had a common origin, but little structural homology capable of leading to pairing remains among the chromosomes. Genetic homologies of 20 of the 21 chromosomes have now been studied, chiefly by testing the ability of tetrasomes to compensate for nullisomes. The 20 chromosomes investigated fall into six groups of three and one group of two, as follows: I, XIV, XVII; II, XIII, XX; III, XII, XVI; IV, XV; V, IX, XVIII; VI, X, XIX; VII, XI, XXI. Tests are under way to determine whether chromosome VIII may belong with IV and XV. It is an obvious inference that each homoeologous group consists of one chromosome each from genomes A, B, and D, the three chromosomes of each group having been derived from the same ancestral chromosome. This inference is supported by the fact that each series contains a single D-genome chromosome (numbers XV to XXI). However, the possibility that additional homologies exist has not yet been excluded, for only 32 of the 420 possible nullisomic-tetrasomic combinations have been studied. Furthermore, since the compensation effect observed in any particular combination probably involves only a few genes, the chromosomes concerned may actually have only a small fraction of their genetic material in common. J4.

SHOWALTER, A. M., Madison College, Harrisonburg, Va. Inbred strains of Cucurbita moschata var. Golden Cushaw, and some hybrids of these inbred strains. — Ten inbred strains, all derived from a common ancestor, appear to be genetically distinct and approximately homozygous as to the principal alleles affecting fruit shape. Numerous crosses were made in 1951 and F_1 hybrids were planted in 1952. D11.

SIEGEL, A. and S. G. WILDMAN, University of California, Los Angeles, Calif. The differential sensitivity of two strains of Tobacco

Mosaic Virus to ultraviolet light. — Two strains of Tobacco Mosaic Virus have been found to differ markedly in their sensitivity to ultraviolet light. A mild strain of TMV, which has been found in natural association with the common strain of TMV, is much more easily killed by ultraviolet light than the common strain. Both strains exhibit an exponential survival curve. Experimental application of the differential UV sensitivity of these strains has indicated that the common strain mutates spontaneously to the mild strain. A purified solution of the common strain has been irradiated to 5% survival, thus insuring the killing of any mild strain particles which might have been present. Single particle infections of tobacco plants with this irradiated solution yield no plants showing mild symptoms. However, virus isolated from a plant infected with a single particle of the irradiated solution is found to contain approximately 2% of the mild strain. This result has been found repeatedly. In no case has the mild strain been observed to mutate to the common strain although the common strain is at a competitive advantage in a tobacco plant infected with a mixture of the two strains under ordinary greenhouse conditions. K4.

SIEGEL, R. W., Indiana University, Bloomington, Ind. The genetic analysis of mate-killing in Paramecium aurelia. — When animals of stock 138 (variety 8 of P. aurelia) conjugate with animals of other stocks, reciprocal fertilization occurs in the normal way; but instead of both exconjugants producing normal progeny, only one does. The exconjugant that receives the bulk of its cytoplasm from the parental cell of stock 138 regularly yields viable progeny, while its mate either dies without undergoing a fission or produces a limited number of daughter cells all of which are doomed to die. Stock 138 is thus a "mate-killer" and the stocks to which it is mated are "sensitives." The existence of mate-killers was previously unknown. — The mate-killer trait is determined by Feulgen-positive cytoplasmic granules called "mu." Mate-killers always possess large numbers of mu particles; cells lacking mu are always sensitive. Loss of mu can be brought about by certain environmental conditions and is irreversible. The maintenance, but not the origin, of mu depends upon a nuclear gene, M. The genetic basis of mate-killing is thus closely parallel to the basis of the killer trait in variety 4. — Mate-killing can be brought about by more or less prolonged contact of a sensitive with a mate-killer cell, but this contact need not involve conjugation. There is no evidence that mate-killers can kill without direct contact. The nuclei of sensitives are damaged, and sometimes destroyed, as a consequence of contact with mate-killers. Similar nuclear damage may occur in the course of the experimentally controlled loss of mu from a mate-killer. The lethal action of mate-killers of variety 8 is in most respects different from that of killers of variety 4. — By virtue of their breeding behavior, mate-killers can receive genes from sensitives during mating; but only under special conditions can the genes of mate-killers be established in a sensitive stock. This predominantly one-way flow of

genes has evolutionary implications and its basis suggests a way in which cytoplasmic factors could play a role in speciation. K9.

SINGLETON, W. R., C. F. KONZAK and D. L. MATTHEWS, Brookhaven National Laboratory, Upton, L. I., N. Y. Microspore and megaspore mutation rates in maize.* — The observable spontaneous microspore mutation rates of the endosperm characters Su, Sh, Wx and Pr appear to be 50 to 100 times higher than the accepted megaspore rates, which are less than 10 per million gametes. The accepted spontaneous rate of change from R → r is approximately 500 per million gametes in the megaspore. The microspore mutation rate for this gene is only about 3 to 6 times higher. — The most plausible explanation for the apparent discrepancy between microspore and megaspore mutation rate is that in the megaspore, only those mutations will be observed that occur before the first nuclear division in the megaspore. There is a period of approximately 24 hours between the first meiotic division and the first nuclear division of the megaspore. Any mutation occurring after this would be cancelled by the non-mutant allele in the nucleus from the opposite end of the megaspore. In the microspore, however, all mutations that occur throughout the period prior to fertilization may be observed. The period from meiosis to fertilization is about two weeks. — There is evidence that the R gene mutates at an earlier time in the microspore development than do the genes Sh, Su and Pr. If such a condition exists in the megaspore, it would explain the "higher mutation rate" of the R gene. Experiments are under way to test this hypothesis in the gamma radiation field this summer. M9.

SNELL, G. D., P. R. F. BORGES, DIANE E. KELTON and SALLY L. ALLEN, Jackson Memorial Laboratory, Bar Harbor, Maine. Histocompatibility genes in the mouse; methods of study, and some results. — Susceptibility and resistance to transplants of normal and tumor tissue are determined by multiple genes. These have been called histocompatibility genes. A detailed analysis of such genes is possible by appropriate genetic methods. These methods include (1) the use of linked genes as markers, and (2) the production of isogenic resistant strains, i.e., strains identical with a known inbred strain except for the substitution at one locus of an allele which produces resistance to tumors transplantable in the inbred strain. Such strains may arise by mutation at a histocompatibility locus, or may be produced by an appropriate series of crosses with testing for, and selection of, resistant animals in alternate generations. By method 1, one histocompatibility locus, called histocompatibility-2, has been analysed in some detail. Five alleles, H-2 (or H-2^{dk}), H-2^{b}, H-2^{d}, H-2^{k} and H-2^{p} have already been described. A sixth allele, H-2^{q}, has now been found in strain DBA/1. Further data have been gathered on the distribution of the

*Research carried out at the Brookhaven National Laboratory under the auspices of the U. S. Atomic Energy Commission.

different alleles in different inbred strains. It has also been found that all hybrids of the genotype H-2^d/H-2^k, even though the parent strains are of quite diverse origin, are susceptible to tumor 1509la which originated in strain A (genotype H-2/H-2). An isogenic resistant line arising by mutation, found by one of us (Borges) in strain C57BL/10, has been shown to be due to a change from H-2^b to H-2^d. Data will also be presented on progress in the production and testing of other isogenic resistant lines. D12.

SONNENBLICK, B. P., Newark College of Arts and Sciences, Rutgers University, Newark, N. J. X-ray exposure in routine diagnostic practice: A survey of 117 fluoroscopes. — Radiation effects in man are influenced by the quality of the radiations, the surface exposed, duration of exposure and rate of administration. Admonitions concerning exposures in diagnostic radiology have long, and with reason, been particularly directed toward fluoroscopic procedures, despite the "softness" of the radiations concerned. That such teachings are still widely disregarded in actual practice was recently ascertained by the writer and three associates in a survey of the roentgen output of 63 fluoroscopes, and of the operator's activities, in New Jersey. The observations were disquieting and a study of 54 more machines in two states was undertaken, with attention focussed on units controlled by pediatricians. Results from the new sample are similar to those initially obtained. — With dials set by the physician operator, the range in output is from 2r to 118r/minute, as measured in air at the panel, with 21% of the 117 units measuring more than 30r/minute. The data collected are, for several reasons, minimal and the skin dose is actually greater than the measured air dose. Physicians in this sample vary decidedly in the use of protective equipment for themselves and in awareness of scatter radiations. Most significant, almost all physicians concerned are completely unaware of the X-ray output of their instruments and few realize the necessity for calibration. Whether a high or low dose is received by the patient seems merely fortuitous. — While fundamental investigations in many laboratories are under way to increase our understanding of radiation effects, concurrent notice of existing radiological practices appears warranted. D13.

SPARROW, A. H., M. DENEGRE and W. J. HANEY, Brookhaven National Laboratory, Upton, New York and Michigan State College, East Lansing, Michigan. Somatic mutations in Antirrhinun produced by chronic gamma irradiation.* — F_1 plants of Antirrhinun majus with dark purple flowers but heterozygous for white, ivory, yellow, intensity and lavender were exposed to chronic gamma irradiation from $Cobalt^{60}$ for several weeks at dose rates varying from a few roentgens to over 300 r per day. In these plants a mutation effecting any one of the above

*Research carried out at Brookhaven National Laboratory under the auspices of the U. S. Atomic Energy Commission.

factors causes corolla cells carrying the mutation to appear as a lighter spot (on a dark purple background). After an adequate period of irradiation, small, light-colored spots or streaks appear on the flowers and produce a characteristic mottling effect. The numbers of such light colored spots were scored on control and irradiated plants from areas of one square centimeter on each superior corolla lobe and one half square centimeter on each inferior lobe. Assuming that each light colored area represents one mutation, it is possible to relate the number of "mutations" produced to dose rate or to total dosage. Graphs showing the relationship between mutation rate and exposure to chronic gamma radiation at several intensities will be presented. E11.

SPIESS, E. B., B. A. TERRILE, and U. BLUMENHEIM, Harvard University, Cambridge, Mass. Wing-beat frequency and wing dimensions as properties of gene arrangement carriers in Drosophila persimilis. — In an attempt to discover properties of third chromosome gene arrangements in D. persimilis from an altitudinal gradient in the Yosemite region of California, strains containing the Whitney and Klamath arrangements from three localities were flown at 20°C in a stroboscope apparatus described by Williams and Chadwick (Science, 1943). Flies to be flown were the F_1's of strain crosses mated in single pairs in half-pint bottles. Flies of the same arrangement from Jacksonville (800′ el.) and Mather (4600′ el.) flew at the same rate: WT/WT averaged 10900 cycles per min., KL/KL 10300, and WT/KL averaged 10700 in both localities. Wing dimensions are larger for KL/KL (3.20 mm^2) than WT/WT (3.10 mm^2) with WT/KL (3.10 mm^2) resembling WT/WT. However, among flies from Mono Lake (6600′ el. on eastern slope of Sierra Nevada) the reverse is the case: WT/WT rate is 10300, KL/KL 10800; wing areas are about equal. Heterozygotes at Mono Lake are not consistent, however, two strain crosses giving 10900 and one giving 10000. Certainly the two gene arrangements differ significantly and can be said to contain different factors concerned with wing-beat frequency and wing dimensions in the localities tested. Apparently the population at Mono Lake is racially distinct for these characters from the populations on the western slope. No evidence was found that wing beat or wing size is correlated with altitude. Also no evidence was found that heterozygotes showed increased vigor for the characters concerned, so that it is doubtful that wing-beat frequency or wing dimensions contribute in any substantial way to the adaptive properties which are known to characterize these gene arrangements. T14.

STALKER, HARRISON D., Washington University, St. Louis, Mo. Diploid and triploid parthenogenesis in the Drosophila cardini species group. — D. parthenogenetica (Stalker, in press) has been maintained unisexually for 15 consecutive generations. 2n ♀♀ of generations 6 to 14 have produced 75% 2n ♀♀, 23% 3n ♀♀, 1.5% sterile XO 2n ♂♂ and 0.5% 2n-3n mosaic ♀♀; total examined: 5138. Heterozygous garnet (sex-

linked recessive) 2n virgins have produced 2n ♀♀ of all three genotypes, but so far only 3n ♀♀ of the genotypes +/+/g or +/g/g, and no mosaics for garnet. Total examined: 572 wild-type 2n ♀♀, 109 garnet 2n ♀♀ and 69 wild-type 3n ♀♀. 3n virgins have produced 1058 flies: 41% XO 2n ♂♂, 30% 2n ♀♀, 29% 3n ♀♀ and 0.4% 2n-3n mosaic ♀♀. No 4n flies have been detected. 2n ♀♀ X 2n fertile ♂♂ produce few or no polyploid daughters, (0/6380 examined). The following provisional explanation appears to fit these data best. Both meiotic divisions are completed forming 4 reduced M-II products. In eggs of 2n ♀♀: fusion of 2 nuclei produces 2n ♀♀, triple fusion 3n ♀♀, non-disjunction of X in M-II plus fusion of 2 nuclei from different secondary oocytes produces XO 2n ♂♂. Activation of egg without nuclear fusion results in haploid embryos and death, (most embryos from unfertilized eggs die prior to hatching). In eggs of 3n ♀♀: fusion of 2 nuclei from different secondary oocytes produces 3n ♀♀; 2n ♀♀ may come from fusion of nuclei from the same secondary oocyte, or, as in the case of XO 2n ♂♂, from unfused 2n nuclei. The 2n-3n mosaic ♀♀ from 3n mothers might result from simultaneous participation of a 3n fusion-nucleus and a 2n unfused nucleus. Similar mosaics from 2n mothers would require some fusion of cleavage nuclei. In the related species, D. polymorpha, the mechanism of parthenogenesis may be similar since of 15 impaternate daughters of 2n ♀♀, 14 were 2n and one 3n. D14.

STEBBINS, G. LEDYARD, JR. and ANTERO VAARAMA, Division of Genetics, University of California, Davis, and State Horticultural Institute, Piikkio, Finland. Chromosome behavior, fertility, and genetic segregation in synthetic allopolyploids between Elymus glaucus and Sitanion. — The F_1 hybrids between Elymus glaucus and either Sitanion jubatum or S. hystrix, all with the chromosome number 2n=28, have nearly regular meiosis with 12-14 bivalents, but are completely sterile. Allopolyploids synthesized from these hybrids by colchicine treatment are fertile, and in later generations segregate for fertility. In morphological characteristics, the C_2 generation is still intermediate, with relatively little segregation in the direction of the morphological characteristics of Elymus or Sitanion. Segregation for pollen and seed fertility occurs more or less independently, with little or no correlation between the two. In nine different allopolyploid plants derived from three different hybrid combinations, the frequency of trivalents and quadrivalents per nucleus ranged from 1 to 7, with means per plant ranging from 4.00 to 5.4. The differences between these means are hardly if at all significant. These data are interpreted to indicate that E. glaucus and Sitanion jubatum or S. hystrix are allopolyploids which have one genome in common, and one in respect to which there is partial homology. If the genome formula of E. glaucus is designated as $A_2A_2E_1E_1$, then that of Sitanion would be $A_2A_2E_2E_2$. The sterility in the original F_1 hybrids is probably due chiefly to cryptic structural hybridity, but also partly to genic factors. Segregation for the latter is

principally responsible for sterility in the allopolyploids of the C_2 generation. B2.

STEIN, K. F. and I. H. ABRAMS, Mount Holyoke College, South Hadley, Mass. The development of mice homozygous for the gene for looped tail. — As reported by Hollander and Strong, the homozygous condition of the gene Lo results in open neural folds, either in the hindbrain only, or in the region extending from this level to the posterior end of the embryo. Examination of histological sections of embryos 9-15 days of age indicates that there is more active proliferation of neural tissue in the hindbrains of these embryos. Approximately one and one-half times as many mitotic figures occur in the abnormal embryos between the last section of the optic vesicle and the first of the otic vesicle as compared with the number found in the same region in normal littermate controls. The effect on the nervous tissue appears to be a localized one. More posterior sections, in the region of the future spinal cord, showed no difference between abnormal and normal embryos in number of mitoses. Also differentiation of notochord and of other structures observed appears to be normal, with the exception that the vertebral arches cannot form in regions of the cord where the neural folds are open. "Loops" were present in the tails of mice homozygous for the gene and in approximately 70% of heterozygotes. The condition involves irregularity in the shape of vertebrae. D15.

STONE, W. H. and JOY E. PALM, University of Wisconsin, Madison, Wis. A disputed parentage case in cattle involving mosaicism of the erythrocytes. — The majority of multiple births in cattle have been shown to involve an intermixture of two or more antigenically distinct types of blood cells derived from genetically different individuals. — This report describes a case in which a cow produced a calf whose blood type contained the blood groups $B^{BO_1Y_2D'}$, A, L and S which could not have come from the sire and which were expressed very weakly or not at all by the dam. Because an individual has to date possessed a blood group only if one or both of its parents had it, this cow would ordinarily be excluded as the dam of the calf. However, her second calf, sired by a different bull, also possessed the blood groups $B^{BO_1Y_2D'}$ and L, neither of which was detectable in the sire's blood. Later, it was discovered that the dam was a twin to a female not available for testing. In subsequent tests, very weak reactions involving unexplained blood groups of the calves were detected in the blood of the dam. The dam's blood was also separated into two distinct cell-types following differential hemolysis. One type, constituting approximately 20% of the cell volume, contained the four blood groups ($B^{BO_1Y_2D'}$, A, L and S) previously unexplained in the blood of her calves and, therefore, must represent the dam's own genotype. The cells in the largest proportion (80%) presumably represent the genotype of the co-twin. — These results show that parentage tests in cattle must be interpreted with caution when twins are involved. B11.

STRAUSS, BERNARD S., Genetics Laboratory, Dept. of Zoology University of Texas, Austin. Pleiotrophic effects of a mutation associated with low carboxylase activity in Neurospora. — The car mutant of Neurospora shows low pyruvic carboxylase activity compared to wild type (car^+). When car is combined with an acetate requiring, glucose inhibited, single gene mutant, ac, to form ac car the glucose inhibition characteristic of ac is suppressed (Arch. Biochem. Biophys., 36, 33(1952)). Crude and partially purified carboxylase preparations from car^+ are indistinguishable kinetically from preparations using car. Crude carboxylase preparations from both strains can be easily distinguished from crude carboxylase preparations from Rhizopus nigricans by their different pH optima and Michaelis constants. Carboxylase activities of both car and car^+ are much higher when these strains are grown on sucrose or glucose than on xylose but the increase in car^+ activity obtained on sucrose is two or three times greater than the increase in car activity. — Low tryptophan desmolase activity is also associated with the car mutation in ten complete asci tested but there is no correlation between the segregation of phosphatase activity and car. Lowered tryptophan desmolase and pyruvic carboxylase activity can be produced by growing Neurospora on a Zn deficient medium but low Zn will not relieve the glucose inhibition of ac car^+ strains. — The effect of the mutation on carboxylase activity is probably quantitative and indirect. G4.

STURTEVANT, FRANK M., JR., G. D. Searle & Co., Chicago, Ill. Correlation between genetic and endocrinologic activities. — From a survey of the pertinent literature, a high correlation appears between the genetic and endocrinologic specificities of several physical agents and chemical compounds. Certain few of the agents which initiate the Alarm Reaction and General Adaptation Syndrome of Selye ("stressors") are capable of inducing thymico-lymphatic atrophy in adrenalectomized mammals, while all other stressors act through the anterior pituitary-adrenal cortex axis. Such "directly" lympholytic stressors include X-rays, neutrons, radium, sulfur and nitrogen mustards, urethane, folliculoids, and desoxypyridoxine, but not colchicine or formalin. The latter two are potent "indirectly" lympholytic stressors, however. All of these agents, with the exception of desoxypyridoxine, have been tested by various workers for mutagenicity and carcinogenicity. Mutagenic characteristics have been reported for the entire group, although the results of experiments on folliculoids are somewhat equivocal and those on colchicine are indicative of stathmokinetic activity only. Carcinogenicity has been established for the radiations and urethane; conflicting data appear for folliculoids and colchicine. — Folliculoids, phenol, radiations, and presumably mustards, urethane, and other radiomimetic chemicals are interpretable as Treffergifte, in the sense of Jordan. — It is suggested that the striking genetic and endocrinologic specificities of this very limited number of agents may be indicative of a similar mechanism of action, viz. by the quantal

release of chemical energy, and thus as Treffergifte. This is not considered evidence supporting the somatic mutation theory of cancer. T15.

TAYLOR, J. HERBERT, Department of Botany, Columbia University. Phosphorus 32 detection by autoradiographs as a method for studying nucleic acid metabolism in individual cells. — Inflorescences of Lilium longiflorum were grown in solutions of phosphate with P^{32} at activities from 20 to 40 μc/ml. At intervals of 8-48 hours after the P^{32} reached the buds, the anthers were fixed and the cells affixed to slides as smears or sections. After extraction of various phosphorus-containing fractions with the appropriate acids or enzymes, autoradiographic stripping film was applied. After exposure the film was developed while still in contact with the cells. Slides were run through an alcohol series and mounted for microscopic examination. — Phosphorus becomes incorporated in the DNA fraction during relatively short periods of the cell cycle. Attention will be given to the microspore mother cells, which undergo meiosis during a period of 5 or 6 days at 75°F. The resulting microspores divide mitotically about 9 days later. In the following 10 days the pollen matures before the generative nucleus divides into the two sperm nuclei. P^{32} is incorporated in the DNA of the chromosomes during a period of 12 hours or less just preceding the onset of meiotic prophase. No further incorporation occurs during meiosis. The next period is of similar duration 36-48 hours prior to the prophase of the microspore division. In the maturing pollen the generative nucleus, but not the vegetative nucleus, incorporates phosphorus about 3 days after the microspore division. These periods correspond to times of DNA synthesis and very likely chromosome reproduction. The timing of synthesis of DNA in the same cells of Tradescantia paludosa follows a similar pattern. The phosphorus turnover in the RNA and phospholipid fractions have been followed in a similar manner. H2.

TEAS, H. J. and E. G. ANDERSON, California Institute of Technology, Pasadena, California. Niacin in starchy and sugary maize. — In comparing kernels from ears of maize segregating starchy and sugary, the niacin content of pericarps, embryos, and inner endosperm was found to be approximately equal. The different total niacin content of su versus Su was due to a greater amount of niacin stored in the aleurone layer of su. When dissected apart aleurone samples from sugary were equal or slightly lower in niacin concentration but were heavier than those from starchy. Measurement of cell size in sectioned kernels confirmed the assay data by showing that the aleurone layer of sugary kernels was about one and one half times as thick as that of starchy. Thus the greater niacin content of sugary is due in the major part to a thicker aleurone layer. M11.

VALENCIA, J. I., Indiana University, Bloomington, Indiana. — Chromosome changes in Drosophila melanogaster spermatozoa induced by mutagenically comparable doses of X-rays and ultraviolet.* Mature spermatozoa were given a rather low dose of X-rays (about 650 r) in order to obtain recessive sex-linked lethals at a frequency (1.8%) comparable with that (1.3%) with which they had previously been produced in spermatozoa by ultraviolet. Detailed cytogenetic analysis showed that 75 of these X-ray lethals included 11 to 12 cytologically visible chromosome changes. These comprised 6 to 7 translocations, 4 inversions (3 gross and 1 of about 60 bands), and only 1 deficiency (about 14 bands). The translocation frequency agrees satisfactorily with the expectation calculated from higher doses but that of inversions appears somewhat larger than expected while that of deficiencies appears smaller than on the hypothesis of both hits involved in such cases being interdependent. The data previously reported (Valencia and McQuate, these Records, 1951) for 70 ultraviolet lethals, produced by the exposures which yielded 1.3% of lethals, had shown only 1 translocation, 1 inversion (gross), and 1 deficiency (of about 18 bands). Allowing for the moderate difference in the mutagenic doses of the two agents, the gross changes appear to be produced more readily by X-rays than by ultraviolet, as had been inferred, although on less direct evidence, from earlier work. The present difference is not yet statistically secure, however. On the other hand, the apparent similarity in frequency of deficiencies at these comparable doses is unexpected. Noteworthy also is the fact that, although the material was carefully scrutinized for very minute deficiencies and inversions, none were found in either series. E12.

VALENCIA, R. M., and J. I. VALENCIA, Indiana University, Bloomington, Indiana. The ineffectiveness of extra heterochromatin in influencing mutation rate in Drosophila melanogaster females.** — In the female controls to an experiment on the X-ray induction of mutations in females, an unusually high spontaneous mutation rate was observed (these Records, 1949). Since in this stock a large Y-chromosome (of sc.Y^{L} type) was present in these females, it was suspected that this might have been the cause of the high mutation rate. This question was then investigated by means of a genetic scheme (to be described), devised by H. J. Muller. In this experiment, spontaneous recessive lethals were tested for in a particular X-chromosome, derived from females ("P_1") some of which ("experimental series")

*Work supported by grants from the American Cancer Society, upon recommendation of the Committee on Growth of the National Research Council, and from the Atomic Energy Commission.

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contained while others ("control series") lacked a sc.Y^{L} chromosome. The experimental and control P_1 females formed numerous small groups of sisters, or sibships, the members of each sibship being divided into equal numbers of females with and without a sc.Y^{L}. Thus the experimental and control P_1 females of each sibship were isogenic with regard to their two X-chromosomes, and their autosomes differed in a purely random manner, the only consistent difference lying in whether the females contained sc.Y^{L}. Cases of lethals arising previously to P_1 were eliminated. The scheme allowed identification of all female combinations of X's and Y's, whether formed by disjunction or non-disjunction. — 3864 experimental and an equal number of control chromosomes were tested. Among the former, 12 lethals (.31%) were found; among the latter, 13 (.34%). It is concluded that, in females at least, the presence of extra heterochromatin results in little or no increase in the spontaneous mutation rate. L5.

VICARI, E. M., Roscoe B. Jackson Mem. Laboratory, Bar Harbor, Maine. Hereditary fatal convulsions prevented by steroid hormones. — It has been shown that the susceptibility to sound-induced seizures at the age level of 28-34 days in the DBA inbred mouse strain is hereditary. Although its precise mode of inheritance is not absolutely clear, there is evidence that multiple factors are involved. In an effort to prevent these fatal seizures five steroid hormones were administered by injection to five different groups of DBA related mice at the high peak of susceptibility. Fifty animals were in each test group. Genetically related mice were used as controls. The results showed testosterone and estradiol effective in reducing the fatal seizures from 96% to 7% and 15% respectively. Pregnenolone reduced them to 30% and cortisone and cortin to 38% and 40% respectively. Previously we found that adrenaline and thiouracil were also effective in reducing fatal convulsions and even prevented the onset of seizure incidence in this same strain of mice. The fact that all these compounds have protective effects suggest that the genetics involved is nonspecific in its physiology. This experimental control of the expression of a supposedly genetic mechanism suggests a sensitive mechanism easily responding to endocrine secretions. C8.

WARD, C. L.* and MARY L. ALEXANDER,* Biology Division, Oak Ridge National Laboratory, Oak Ridge, Tennessee. A cytological analysis of X-ray-induced mutations in Drosophila melanogaster. — A cytological study of X-ray-induced mutations has been undertaken to determine the proportion of point mutations to those mutations which are associated with various types of structural changes of the chromosome. The mutations at eight specific loci in the third chromosome, ru h th st p^{p} cu sr e^{s}, were obtained by Alexander from irradiated sperm and spermatogonia. Of the 21 mutations thus far studied, 10

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have been classed as point mutations (no visible chromosomal aberration), 3 are associated with deletions, 5 with inversions of various sizes, and 3 with translocations. All inversions are large rearrangements except for one at the pink locus which involves a maximum of 15 bands. Seven of the 10 point mutations are viable in the homozygous condition while three are lethal or semilethal. The deletions are lethal in the homozygous state, and four of the five inversions live as homozygotes. — A cytological analysis of the offspring of an individual showing two mutations, thread and scarlet, reveals a deletion of approximately 25 bands in regions 72 and 73A of the left arm of the third chromosome. The deletion includes the region which has been assigned to thread by an earlier investigator but not that which has been assigned to scarlet. Analyses of other scarlet mutations show a deletion of the regions 72F and 73A and a translocation with the break in region 73A. These three chromosomal abnormalities indicate that the scarlet gene is located in either 72F or 73A of the third chromosome. A13.

WEIR, J. A., University of Kansas, Lawrence, Kansas. The nature of genetic resistance to infection in mice. — Selection for leucocyte count, through 9 generations of mice, combined with inbreeding, has produced high and low lines with means of 14,495 ± 430 and 5729 ± 273 cells per cmm. Means for random bred controls were 9580 ± 278 at the outset and 9482 ± 308 eight generations later. Twenty one F_1 mice had a mean of 6287 cells indicating dominance of low count. The brown locus is associated with leucocyte count and Bb and bb mice have lower counts than BB mice. — Mice of the ninth generation inoculated with 200,000 live organisms of Salmonella typhimurium had survivorships of 27% for the high line, 43% for the lows and 49% for random bred controls. Results are consistent with 5 previous tests involving 1200 mice in all. High count mice are also susceptible to toxic effects of killed organisms. — Selection for blood pH has been successful in separating out a high line with pH 7.47 and a low line with pH 7.42. Tested in the same manner as the leucocyte strains, high blood pH mice proved relatively susceptible with survival of 29% contrasted to 40% for lows and 42% for random bred controls. — These results, together with those of Hill et al. — J. Hyg. 40: 538-547 (1940)—concerning susceptibility of toxin resistant mice to live organisms, were unpredictable in view of the Iowa State College findings that inbred resistant strains are characterized by high leucocyte count, high blood pH and resistance to toxin. Evidently these mechanisms do not act as simple additive components in the resistance complex. C9.

WHITING, ANNA R. and R. C. von BORSTEL, University of Pennsylvania, Philadelphia, Pa. Comparison of sensitivity to nitrogen mustard and x-rays of Habrobracon eggs and sperm.* — Adults were

*This investigation was supported by a research grant from the National Cancer Institute, of the National Institutes of Health, Public Health Service, to the senior author.

treated with a 10% solution of methyl bis (beta chloroethyl) amine hydrochloride in alternating exposures of forty seconds aerosol and ten seconds air. Lethal dose for eggs in first meiotic metaphase (metaphase I) is about ten minutes. No treatment up to one and one half hours was lethal to all eggs in first meiotic prophase (prophase I). An exposure of one half hour was lethal to sperm in preliminary experiments. There is no recovery in metaphase I eggs or in sperm. — Approximate lethal x-ray doses are, for metaphase I 2000r, for sperm 10,000r and for prophase I 50,000r. Lethal exposures in minutes to nitrogen mustard on this basis should be 10 : 50 : 250. More adequate tests of sperm may confirm this expectation. Complete prophase I lethality will perhaps be unattainable due to sensitivity of females. — In controls one third of eggs laid by mated females develop parthenogenetically into males. When males were heavily treated with nitrogen mustard and mated immediately to untreated females, hatchability of eggs in metaphase I was greatly reduced (2/210 or 0.95%; expected at least 70/210 or 33.33%). When heavily treated females were mated at once to untreated males all progeny were male. The exposed individuals of each sex can induce injury to gametes of opposite sex during mating. In later experiments all exposed individuals were kept for several hours before mating. A1.

WILSON, G. B., TeMAI TSOU and PETER HYYPIO, Dept. of Botany, Michigan State College, East Lansing, Mich. Variations in Mitosis. — An attempt is being made to systematize deviations from "normal" mitotic behaviour in terms of variability in spindle action. It is hoped that such attempts at systematization will throw some light on the type or types of forces concerned with chromosome movement. The pertinent data and illustrations have been obtained largely from Allium root tips treated with a variety of organic compounds especially colchicine and cyclochlorohexane. It is noted that all of the deviations described may be found in untreated tissues either regularly or sporadically depending on the tissue and the deviation. H1.

WOODS, PHILIP S.* and ROBERT E. DUNCAN, Program in Cytology, Department of Botany, University of Wisconsin. Cytological effects of benzimidazole and 5-aminouracil. — Onion roots were immersed for up to 48 hrs. in aqueous solutions of 150, 300, or 600 p.p.m. of benzimidazole or 31, 63, 125, 250 or 500 p.p.m. of 5-aminouracil at pH 5.8 ± .3. These and control roots growing in aerated glass-distilled water were kept in the dark at 22 ± .5°C. Within 24 hrs. both compounds decrease the mitotic index, the higher the concentration the lower the index. Mitotic activity ceases by 24 hrs. in roots immersed in 600 p.p.m. of benzimidazole. At the higher concentrations, benzimidazole produces a somewhat greater contraction of chromosomes than normal, but no irregularities in spindle function or chromosome

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disjunction. — 5-Aminouracil produces Feulgen negative regions in the chromosomes but does not destroy their unity in that particular division cycle. These regions become elongated in anaphase. By 24 hrs. there are fewer micronuclei than would be expected if fragmentation occurs and one or more results from each such anaphase. — On the assumption that these two analogues of the purines and pyrimidines of nucleic acid, respectively, interfere with nucleic acid metabolism photometric determination of the DNA content of energic nuclei at 24 hrs. were made. The relative frequency of 2C and 4C nuclei in control and benzimidazole treatments do not differ greatly. Roots treated with 5-aminouracil, however, contain few nuclei with the 4C amount. Apparently 5-aminouracil prevents doubling of DNA content whereupon divisions cease. It is thought to specify that one activity of 5-aminouracil is interference with the accumulation of DNA within the energic nucleus. Stoppage of mitosis by benzimidazole needs further analysis. T16.

WOODWARD, V. W., J. R. DE ZEEUW, and ADRIAN M. SRB. Cornell University, Ithaca, N. Y. Selection of Particular Biochemical Mutants in Neurospora from Differentially Germinated Conidial Filtrates. — A major problem in biochemical genetic technique is to separate particular mutants from the other various genotypes occurring in a population of nuclei subjected to mutagenic treatment. Particular mutations in Neurospora which may block a pathway in metabolism can be efficiently isolated by a method involving filtration and selective plating. Conidia treated with a mutagen are incubated under forced aeration in a minimal medium. Under these conditions, germination of wild type conidia occurs. The resulting mycelium is removed at twelve hour intervals by aseptic filtration through two layers of cheesecloth. After 5-7 days, most of the spores which can germinate on minimal medium have been eliminated, and a concentrate of mutants remains behind. The conidial filtrate is plated onto minimal agar medium supplemented with a specific substance which will select desired biochemical mutants. (Sorbose is added to the plates to induce colonialization.) Among experiments conducted, arginineless mutants were sought, utilizing this discriminatory germination technique. Of the isolates made, 20.3% had growth requirements for arginine. G5.

YAMASHITA, K., Kyoto University, Kyoto, Japan and State College of Washington, Pullman, Washington. Synthesis of all types of ring complexes in Einkorn wheats. — Reciprocal translocations (RT's) induced by X-ray treatments of dormant seeds of Einkorn wheats, Triticum aegilopoides and T. monococcum, were analyzed and their chromosome constitutions established. In an attempt to combine the chromosomes into larger rings, successive crosses among the established RT-types have been made. Consequently all possible combinations of rings and pairs obtainable from the 14 Einkorn chromosomes have been realized, giving a series of structural hybrids comparable with the well

known situation in Oenothera. In one of the cross combinations, the crosses were so planned that the chromosomes of T. monococcum associate with those of T. aegilopoides as a (14) = monococcum/aegilopoides complex heterozygote. — The frequency of zig-zag figures in the associations of four chromosomes amounted to 83% on an average, which approximated the pollen fertility of 91% on an average. The fertility of other synthesized plants with different size of rings and pairs of chromosomes were found to be as follows: (4) + (4) + 3_{II} = 81%, (4) + (4) + (4) + 1_{II} = 68%, (6) + 4_{II} = 76%, (6) + (4) + 2_{II} = 71%, (6) + (4) + (4) = 35%, (6) + (6) + 1_{II} = 51%, (8) + 3_{II} = 49%, (8) + (4) + 1_{II} = 40%, (8) + (6) = 37%, (10) + 2_{II} = 40%, (10) + (4) = 20%, (12) + 1_{II} = 30%, and (14) = 13%. The two species and their hybrids with 7_{II} were fully fertile. J6.

YANDERS, A. F., University of Nebraska, Lincoln, Nebr. X-ray induced chromosome aberrations and dominant lethals in Drosophila robusta.* — Newly emerged males and females were separated for 10 days prior to treatment of the males with doses of X-rays of 5,000, 7,500, and 10,000 r units. The percentage of dominant lethals induced in the sperm of the irradiated males was determined by comparisons with non-irradiated controls of offspring resulting from eggs laid within ten days after irradiation. Some of the progeny of the groups treated with 5,000 r units were dissected as larvae for the detection of induced chromosome aberrations. At this dose, a much smaller percentage of induced chromosome aberrations was recovered than anticipated from published results with other species. Slightly higher values for induced dominant lethals were obtained in all groups, although the trend of reduction of offspring number closely parallels that in other species. Since it has been shown in D. melanogaster that dominant lethals are induced more easily in sperm of aged males and immature germ cells than in sperm which has just matured, with a corresponding decrease in viable aberrations, it seems most probable that both the higher values for dominant lethals and the lower ratio of recoverable chromosome aberrations in D. robusta are due to different responses to irradiation of germ cells of different physiological age. D16.

YERGANIAN, GEORGE,** Biology Department, Brookhaven National Laboratory, Upton, L. I., New York. Cytogenetic possibilities with the Chinese Hamster, Cricetulus barabensis griseus.† — In 1943, Pontecorvo erroneously reported this species to possess a haploid number of 7 chromosomes. Recently, Matthey, Sachs, and the writer found it to have 11 pairs of chromosomes. The Old World members

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of the sub-family Cricetinae, or "Dwarf and True Hamsters," are characterized cytologically by large sex bivalents. Attempts to induce autosomal and sex-linked mutations among the 11 genetic linkage groups are now in progress. The very late pachytene chromosomes are easily stained in squash preparations which should facilitate detecting induced chromosomal aberrations. At this stage, the sex bivalent is a heterochromatic mass measuring 5 x 8 microns, and the autosomes vary from 6 to 33 microns in length. The spherical nucleolus, attached to the sex bivalent, has a diameter of 5 microns. The nucleolar organizer is heterochromatic and in the shape of a half-sphere having a diameter of 3 microns. A brief review of the problems encountered while breeding the animals, and planning the diet will be presented. The possibility of inducing tumors by treatment with methylcholanthrene and 9,10-dimethyl-1,2-benzanthracene is also being investigated. If the above trials are successful, histocompatibility tests between genetically different ascites tumor cells and hosts will be initiated. In addition to this, cytological observations involving the 11 pairs of morphologically different chromosomes in ascites tumor cells will be possible. H3.

ZINDER, N. D. and J. LEDERBERG, University of Wisconsin, Madison, Wisconsin. Gene transfer in Salmonella. — A new mechanism for factor transfer has been found in the bacterium, Salmonella typhimurium. This mechanism is fundamentally different from sexual recombination as found in the bacterium, Escherichia coli and it shares some aspects with the "type transformations" of the pneumococcus and Hemophilus. We have called this process transduction, which is defined as genetically unilateral transfer to stand in contrast with the union of equivalent elements in fertilization. — When S. typhimurium is attacked by "lysogenic" phages, it releases a filtrable agent (FA) which can transfer individual genetic traits from one bacterium to another. In detailed experiments wherein the FA-donor and the FA-recipient differed in a number of different characters, each character was subject to transduction but each independently of the others. — Such characters as nutrition, fermentation, drug resistance and antigens have been transduced. The character transduced is stable both after vegetative reproduction and iterated transduction. — It is tempting to identify the active material as bacterial genes, at best small chromosomal fragments which undergo a kind of crossing-over with the host's chromosomes. — FA can be identified with a heat stable chloroform resistant particle about one-tenth micron in diameter. It is stable to desoxyribonuclease and so differs at least superficially from the transforming principles of pneumococcus and Hemophilus. However, much evidence has been accumulated implicating the bacteriophages, once thought of as genes themselves, as passive carriers of the genetically active material. — Salmonellae having somatic antigen XII adsorb S. typhimurium FA and some inter-type transfers have been accomplished. These latter include some antigenic hybrids indicating that transduction might play a role in the evolution of new serotypes in this x antigenically complex group. I6.